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THE TIME AND MODE OF DUPLICATION OF CHROMOSOMES*

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Impressions gained from limited evidence and reinforced by repeated observations are sometimes difficult to correct even in the face of overwhelming data to the contrary. The situation is illustrated by the ideas about the changes in chromosomes during cell division. During the past six or seven years abundant evidence has been accumulated to show that the deoxyribonucleic acid (DNA) content of chromosomes does not change during the stages of division. Yet, the general awareness and acceptance of this fact has been slow in coming. Let us examine briefly some evidence that produced the impression of increasing amounts of DNA in division stages.

CHANGES IN AMOUNT OF DNA DURING MITOSES

For years biologists had observed the changes in prophase that produced dark staining chromosomes from the diffuse material of interphase nuclei. The eye is very sensitive to differences in contrast of objects in a microscopic field, but even a highly trained observer finds great difficulty in correcting for shape and volume changes in comparing objects of different contrast. Feulgen's method of staining DNA showed that the condensed chromosomes had a high concentration of this material. The impression that it was increasing during the prophase condensation of the chromosomes followed easily. In addition, the impression that materials including DNA were accumulating on the chromosomes during prophase was supported by the first attempts at quantitative determinations at the cellular level. Caspersson (1939) calculated, from measurements of ultraviolet absorbing

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materials in grasshopper spermatocytes, that nucleic acids increased from 16 to 26 $\times 10^{-9}$ mg per nucleus between early prophase and metaphase. In later papers he also calculated that the ratio of nucleic acids to protein changed from 1:20 in early prophase nuclei to about 1:3 in metaphase chromosomes (Caspersson, 1940a; 1940b; and 1950). Since his method did not distinguish between RNA and DNA and the latter result could be attributed to changes in the amount of protein, these findings do not prove an increase in DNA during prophase. Although Caspersson was usually cautious in making interpretations, other less cautious workers took his suggestions and on the basis of observations unsupported by quantitative data proposed various hypotheses concerning accumulation and discharge of DNA from the chromosomes and the relation of these presumed changes to coiling, pairing and crossing-over.

The first cytophotometric measurements of Feulgen dye content of dividing onion root cells and grasshopper spermatocytes also indicated an increase of DNA during prophase (Ris, 1947). Although these early results were preliminary and were soon corrected, they served to reinforce ideas already widely held. To further complicate matters, contradictory results, sometimes utilizing similar methods and materials, continued to be reported.

Following the work of Boivin et al. (1948) and Mirsky and Ris (1949) which indicated that diploid somatic cells had an average amount of DNA almost exactly two times that of haploid sperms, the cytophotometric method began to be used to study changes in DNA content of nuclei accompanying cell division (Swift, 1950a and 1950b; Alfert 1950; Pollister et al. 1951). From these studies it was clear that the amount of DNA had doubled by prophase in cells of somatic tissues in which divisions were occurring. Early prophase nuclei had two times as much DNA as late telophase nuclei. Interphase nuclei contained amounts varying from the telophase amount to the prophase amount.

In the meiotic divisions DNA synthesis was essentially complete by the beginning of meiotic prophase (leptotene) in spermatocytes of the mouse (Swift 1950a). In Tradescantia the DNA appeared to double during leptotene but synthesis certainly was complete by the end of zygotene. Alfert's (1950) results showed that the increase of DNA in the mouse oöcyte had occurred by the earliest stage at which the cells could be recognized, although he did not indicate the stages of meiotic prophase.

TIME OF SYNTHESIS OF DNA

Evidence was soon available from another source. Several biochemical studies had shown that radiophosphorus was incorporated into DNA of different tissues roughly in relation to the number of new cells formed and once incorporated, turnover occurred at exceedingly low rates. Howard and Pelc (1951a) applied the isotope technique to individual cells of the roots of *Vicia faba*. After allowing the roots to grow in phosphorus-32 for an interval, the cells were fixed and hydrolyzed to remove non-nucleic acid phosphorus and RNA. Then stripping film was applied and autoradiographs

prepared. By counting the number of metaphases that had incorporated P-32 they could show that prophase and metaphase chromosomes did not incorporate the phosphorus into DNA. In fact their results indicated that on the average about 8 hours intervened between the cessation of incorporation and metaphase. Since the mitotic cycle (metaphase to metaphase) in these cells is about 24 hours, the synthesis of DNA occurred during middle interphase and appeared to extend over a period of about 8 hours.

Confirmation that DNA synthesis was limited to interphase also came from another study (Walker and Yates, 1952) which had the added advantage that the history of each nucleus measured could be traced from photographs that were made at intervals before the cytophotometric measurements. Therefore, the approximate stage of interphase at which the measurement was made could be determined. They measured the total amount of ultraviolet absorbing material in living cells and then fixed comparable cells and measured the DNA (Feulgen) per nucleus. They found that early interphase nuclei in tissue culture had approximately double the amount of ultraviolet absorbing material as comparable diploid non-dividing cells. This post-telophase amount was again doubled during interphase, but reached its maximum before prophase. Furthermore the metaphase chromosomes had a total absorption less than that of the pre-prophase interphase nucleus. Measurements of DNA (Feulgen) indicated that synthesis occurred during interphase and that the amount of Feulgen stainable material in post-telophase nuclei was equivalent to that in non-dividing nuclei, for example, erythrocyte nuclei.

DNA DURING MEIOSIS

About the same time we had obtained data by means of autoradiographs that allowed more exact determination of the stage of incorporation of P-32 into DNA of Lilium and Tradescantia during meiosis and the subsequent mitoses in the microspore and pollen grain. The advantage here was that microgametogenesis extends over a period of 16–17 days in Tradescantia and more than 40 days in Lilium. Incorporation was determined for intervals of 12–24 hours during microgametogenesis (Taylor, 1952 and 1953). In Lilium incorporation of phosphorus into DNA ceased well before leptotene. In Tradescantia the synthesis extended into what is usually called early leptotene, but ceased well before zygotene pairing began. At no other time during meiosis was there any incorporation of phosphorus into DNA of microsporocytes, even though incorporation might be occurring vigorously in the adjacent tapetal cells.

In both Tradescantia and Lilium incorporation of phosphorus into DNA of microspore nuclei occurred at late interphase, occupying only one fifth or less of the long interphase. In the two-nucleate pollen grain the period of synthesis was about the same length, but in this case occurred during middle interphase.

The autoradiographic findings confirmed the cytophotometric results and

in addition served to locate more precisely the stage during which synthesis occurred. The autoradiographs also showed what could not be proven by the cytophotometric methods as then used, that is, that no extra synthesis, beyond the doubling that had occurred in interphase, was involved in the late stages of mitosis and meiosis. Later when a two-wavelength method was devised (Patau, 1952; Ornstein, 1952) which made measurements of the amount of DNA in condensed chromosomes possible, cytophotometric data confirmed this point (Patau and Swift, 1953). Groups of metaphase chromosomes were found to have the same amount of DNA as prophases. The coefficient of variation was only 5.5 percent.

To test the assumption that incorporation of P-32 into DNA was regularly correlated with a net increase of DNA, studies were carried out utilizing the autoradiographic and cytophotometric methods simultaneously (Taylor and McMaster, 1954 and Moses and Taylor, 1955). The two events occurred simultaneously in every instance investigated. This included the syntheses at premeiotic interphase, late interphase in the microspores, middle interphase in the 2-nucleate pollen grains of both Lilium and Tradescantia, as well as the interphases preceding the two mitotic divisions that occur in the tapetal tissue of Lilium.

Several additional studies have made it clear that DNA synthesis regularly occurs in interphase when the chromosomes are uncoiled or very loosely coiled. However, the part of interphase occupied by DNA synthesis varies with the organism and the type of tissue involved.

DNA synthesis in grasshopper neuroblasts was determined by following the incorporation of thymidine-C-14 (Gaulden, 1956). This study is especially significant since thymidine is incorporated exclusively into DNA and the cells used could be observed continuously in hanging drop cultures. The individual cells were watched and fixed at any desired stage after incorporation began. Synthesis begins in middle telophase and continues through the entire interphase into very early prophase. However, account should be taken of the rather unusual classification of stages in these living cells (Carlson and Hollaender, 1948). The middle and late telophase and very early prophase would be classified as interphase by most cytologists. Maximum uptake occurred during late telophase and interphase which together occupy less than one third of the mitotic cycle.

The only other report of the synthesis of DNA in telophase is one by Pasteels and Lison (1950) who concluded from cytophotometric measurements of DNA (Feulgen) that synthesis was completed during telophase in erythroblasts of rat embryo, Lieberkühn glands of adult rats and fibroblasts of chicken heart in tissue culture. These results were apparently due to technical errors in photometric measurements (see Alfert and Swift, 1953) and perhaps in part to the criterion used for classification of stages in these rapidly dividing tissues where duration of the various stages were not given.

Our study of *Tulbaghia violacea* (Liliaceae) by the use of phosphorus-32

and the autoradiographic technique (Taylor, 1956) has shown that the timing of DNA synthesis in this species follows the pattern typical of Lilium and Tradescantia with one significant difference. The synthesis in the microspore interphase occurs at the very beginning of the long interphase rather than its end. DNA synthesis occurs in the generative nucleus of the two nucleate pollen in mid-interphase. In the pollen grains the tube nucleus of Tulbaghia and Lilium does not appear to double its DNA, but in some forms of Tradescantia an increase appears to occur (Moses and Taylor, 1955). This difference in Tradescantia may be related to the fact that one mutant of Tradescantia has a high frequency of pollen grains in which the tube nucleus divides in the maturing pollen grain (Anderson and Steffensen, 1956).

RESTRICTION IN TIME OF DNA SYNTHESIS

Why is DNA synthesis restricted to a part of the cell division cycle? If an answer can be obtained, it may provide a further clue to the role of DNA in cellular function. The condensed condition of the chromosomes in the late division stages might prevent their duplication and the consequent DNA synthesis, but why should the synthesis be restricted to a part of the interphase or at least to a part of the cycle during which the chromosomes are in the extended condition? We first noticed (Taylor and McMaster, 1954) that the periods of peak synthesis of RNA and DNA in cells did not coincide. More recently (Taylor, 1956) with better methods for differential extraction of RNA and DNA phosphorus in tissue sections, a separation of the two processes in time was more clearly shown. As mentioned above the synthesis of DNA in the microspore of Tulbaghia occurs at early interphase. Since both RNA and DNA synthesis stops in the late stages of meiosis, it is possible to expose cells to P-32 for a period before the beginning of synthesis of DNA and to fix them before synthesis is complete. Such cells would have no chance to incorporate P-32 into RNA unless the incorporation occurred simultaneously with DNA synthesis. In these cells the results indicate that the two events were definitely separated in time. RNA synthesis began toward the end of DNA formation or very soon after DNA synthesis was complete. Other examples are not so clear cut and more examples are necessary before a decision can be made as to the regularity of the separation of the two processes.

DNA AND DUPLICATION OF CHROMOSOMES

A problem that interested us from the very beginning in this work was the relation between DNA synthesis and chromosomal duplication. The answer has important implications with regard to the mechanisms of self-duplication and crossing-over. When the results on the time of synthesis of DNA were mentioned before a group of geneticists at the Cold Spring Harbor Symposium in 1951 and the suggestion was made that the two events were the same, objection was made that the two events need not coincide

(see the discussion following the paper by Schultz and Redfield, 1951). The objection was raised because chromosome duplication must occur after zygotene pairing to support Belling's (1931) hypothesis of crossing-over. Schultz pointed out that the DNA of the new chromatids might indeed be synthesized before pairing occurred but that the interchromomeric fibers might be formed after pairing. Although this seemed unlikely, there was then insufficient evidence to the contrary. That evidence is available now and I believe Belling's hypothesis, and all others that require chromosomal duplication after zygotene pairing, can be consigned to the list of untenable hypotheses. The Belling hypothesis is ruled out by both the timing of chromosome duplication and by the mode of duplication (see the section below on tritium-labeled chromosomes).

Another approach to the problem of chromosomal duplication was to determine the time of synthesis of the chromosomal proteins in relation to DNA synthesis. Howard and Pelc (1951) first reported that protein synthesis, as measured by uptake of sulphate-S-35 into chromosomes, occurred at the same period of interphase in Vicia as DNA synthesis. We showed the same thing for meiosis in Lilium (Taylor and Taylor, 1953). However, these results were not considered conclusive, for some incorporation occurred in the nucleus during meiotic prophase. Cytophotometric data of Alfert (1955) and Block and Godman (1955) showed that during mitotic interphase the synthesis of DNA and basic proteins occurred simultaneously.

A more direct approach to the problem is the relation between DNA synthesis and the duplication of chromosomes as revealed by X-ray breakage. Originally, we pointed out the correlation between the period of DNA synthesis in the microspore and pollen of Tradescantia (Taylor, 1953 and Moses and Taylor, 1955) and the carefully determined time at which the chromosomes become double to X-ray breakage (Sax, 1941 and Bishop, 1950). In addition our preliminary results with Tulbaghia show that chromatid type aberrations occur with a high frequency during most of the microspore interphase as would be expected since DNA synthesis occurs in early interphase. Thoday (1954) has shown that his data on the change from chromosome to chromatid type aberrations coincides with the period of DNA synthesis in Vicia root cells as determined by Howard and Pelc (1951). Finally, Mitra (1956) has shown that the two events coincide in cells about to enter meiotic prophase, that is, at late premeiotic interphase in microsporocytes of Lilium.

A question that remains concerns the organization of the DNA in the chromosome, its relation to the other components of the chromosome and its mode of duplication. Although we are far from final answers, our recent experiments (Taylor et al., 1957) in tracing chromosomes labeled with tritiated thymidine give some significant clues. The radioactive isotope of hydrogen, tritium, emits beta particles of very low energy. For this reason it gives the best resolution obtainable in autoradiographs. Labeled and non-labeled parts of individual chromosomes were resolved. The

tritium was introduced into the DNA of the cell by growing seedlings in solutions containing tritium-labeled thymidine which we prepared by catalytic exchange. Thymidine has the unique property of being a selective and specific label for DNA (Reichard and Estborn, 1950, Friedkin et al., 1956 and McQuade et al., 1956). When fed in low concentrations to cells of rapidly dividing tissues it is incorporated into DNA almost as fast as it enters the cell. When seedlings are removed from the isotope solution only a very small pool of labeled precursor remained in the cells. This was quickly depleted and synthesis of DNA continued in the absence of the tritium-labeled precursor. Only the DNA synthesized when the seedlings were growing in the isotope solution and for a short time after removal was labeled. Since DNA has a very low turnover rate, this DNA remained labeled and no additional molecules became labeled. The labeled DNA was built into chromosomes and the question of its future behavior was answered by observing the descendents of the labeled chromosomes. The number of generations or replications of the labeled chromosomes was determined by transferring the seedlings to a colchicine solution which blocked cell divisions but did not prevent chromosome duplication. The ploidy (number of sets of chromosomes) observed gave the number of duplications since labeling. Chromosomes in cells with 12 chromosomes, the usual number, had not duplicated since they were labeled. Chromosomes in cells with 24 or 48 had duplicated one and two times, respectively.

When no duplication intervened, all chromosomes were uniformly labeled. Each of the two daughter chromosomes (chromatids at c-metaphase) were equally labeled (figure 1). After one duplication these labeled chromosomes produced a labeled and a non-labeled daughter chromosome. The only exceptions were chromosomes in which sister chromatid exchange had occurred. In these, complementary portions of the two chromatids were labeled. After two duplications had occurred in the absence of labeled

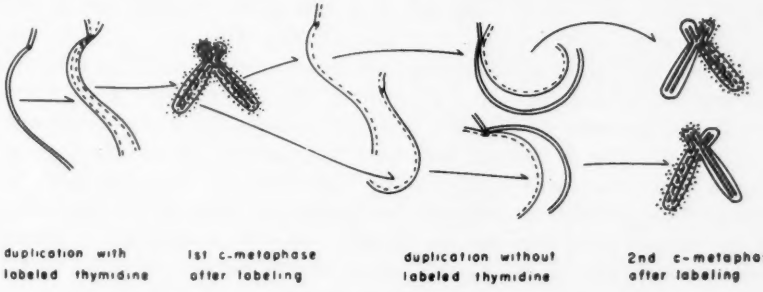

FIGURE 1. Diagrammatic representation of proposed organization and mode of replication which would produce the result seen in the autoradiographs. The two units necessary to explain the results are shown, although these were not resolved by microscopic examination. Solid lines represent non-labeled units while those in dashed lines are labeled. The dots represent grains in the autoradiographs (after Taylor et al., 1957).

precursors, about one fourth of the daughter chromosomes were labeled. Therefore, except for the sister chromatid exchanges no further distribution of the labeled units occurred.

MODE OF DUPLICATION OF CHROMOSOMES

We interpret these findings to mean that the chromosomes before duplication are composed of two units which extend throughout the length of the chromosome (figure 1). The units separate at duplication and each has a complementary unit built along it. Each pair of complementary units appears as a uniformly and equally labeled daughter chromosome at the following division. However, each chromosome must be composed of an original non-labeled unit and a new labeled unit as revealed by its subsequent behavior. At the next duplication the labeled unit separates from its non-labeled complement and has a non-labeled unit built along it. This results in a labeled daughter chromosome (chromatid at c-metaphase). The other unit must not have been labeled, for it produces a daughter chromosome completely free of label.

The mode of duplication is analagous to the scheme proposed by Watson and Crick (1953) for the two complementary polynucleotide strands of the DNA double helix. However, the chromosome is several orders of magnitude larger than the DNA double helix and we do not know whether the units which we have discovered share strands of the DNA double helix or not. Several working models for the chromosome have been constructed but considerable additional information is necessary to choose among them.

Models of the duplication process. Three models will be considered briefly. The first is that the axis of the chromosome consists of one long strand of DNA, a Watson-Crick double helix. The major difficulty with this model, or any of its variations, is the tremendous length of the piece of DNA and the mechanical problems of unwinding the two strands of the double helix. In Lilium where we know the amount of DNA per haploid complement to be about 53×10^{-12} gm. of DNA (Ogur, et al. 1951), the length can be calculated. The length for the complement would be 1.5×10^{7} microns (15 meters) with 4.41×10^{9} coils of the double helix. For each of the 12 chromosomes the length would vary between one and one and one-half meters. The mechanical and energetic problems of unwinding a long double helix have been considered by Levinthal and Crane (1956). Although their calculations indicate that unwinding would not be impossible if the double helix were not too sharply folded or coiled, the remaining problem of folding or coiling this strand into a structure with the dimensions and mechanical properties of a chromosome is a formidable one.

A second model considered by Taylor et al. (1957) is a multistranded chromosome consisting of two complementary multistranded ribbons. A ribbon has the desired mechanical properties for coiling at the microscopic level if built so that the middle and edges contract at different rates (figure

2). When differential contraction occurs the ribbon will roll into a trough-shaped strand and assume the form of a helix. If the ribbon were not uniform along its length because of variations in either the middle or edges of the ribbon, the helix would be irregular and therefore would present the aspect of chromomeres. A second or even a third set of coils could develop in such a structure as a result of the torsions produced by differential contraction of its parts. Although attractive for its mechanical properties this model can not explain the separation of many longitudinally oriented intertwined double helices in any way that would segregate the old from the new DNA. If the model were accepted the scheme of duplication of DNA proposed by Watson and Crick could not be supported by our findings. Therefore, a variation of the ribbon will be considered.

The third model consists of a ribbon in which the DNA is oriented at right angles to the axis of the chromosome and attached in a regular way to a central core or axis (figure 3). The core is visualized as composed of two ribbons lying together with DNA double helices attached only at their edges in such a way that one polynucleotide chain of each double helix is attached to one ribbon of the core and the other polynucleotide chain to the other half. The other end of the double helix would be free to rotate at least during synthesis of DNA. Synthesis could be initiated by the

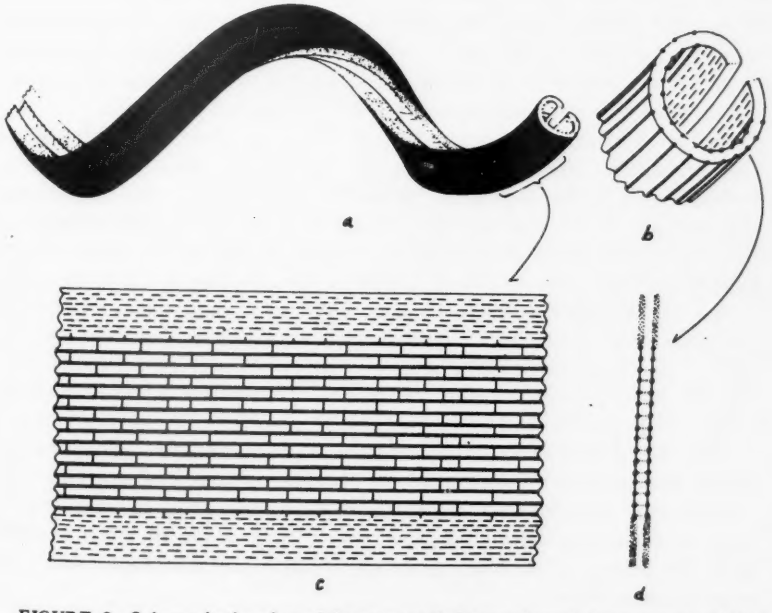

FIGURE 2. Schematic drawing of the proposed ribbon-shaped chromosome with the two multistranded units folded together and coiled; (a) a small sector from the coiled chromosome; (b) detail in cross-section; (c) a short sector of the multi-stranded units in longi-section, uncoiled and flattened; (d) cross-section. The number and size of strands shown have no special significance (after Taylor et al., 1957).

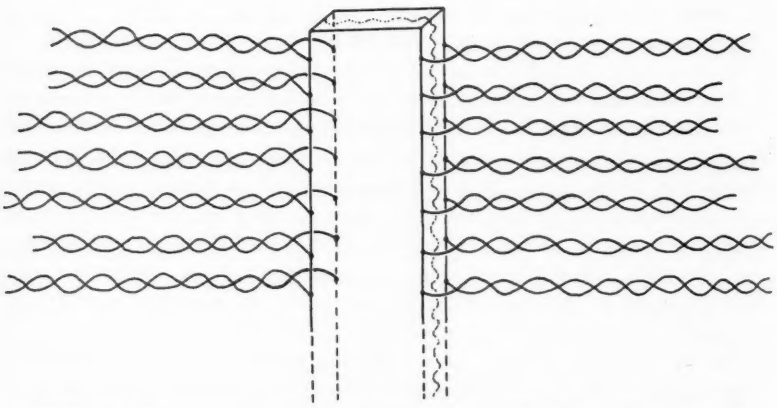

FIGURE 3. Diagrammatic sketch showing the end portion of the chromosome axis as proposed in the third model. The nature of the axis is unspecified, but the side branches represent Watson-Crick double helices of DNA.

separation of the two elements of the core with consequent breakage of some proximal hydrogen bonds in the double helix. The single bonds in each polynucleotide chain should then allow rotation and with the other end of the double helix free to rotate, separation could be achieved without mechanical difficulties. The polynucleotide chains could replicate as proposed by Watson and Crick. All of these new polynucleotide chains would be attached to the new half of the core of each daughter chromosome and therefore would be passed on as a unit in future replications.

Recent electron micrographs (Moses, 1956 and Fawcett, 1956) show a core of electron dense material along the chromosome axis, at least at certain stages in the cell cycle. However, the structures photographed are several times as thick as the central axis proposed here. In this model the attachment sites must be spanned by a single double helix. Some type of folding could account for the thickness at certain stages, but to speculate on the organization or composition of the core is probably not fruitful at this time. The model only requires that the two ribbons of the core have repeating units or sites opposite each other for the attachment of the DNA. The two halves could be complementary but perhaps the structural requirements are no greater than for any other laminated structure in the cell.

This third model is not only attractive for its mechanical properties, but from a genetic point of view another dimension is added for crossing over. Conventional crossing over might involve exchanges along the axis or core, while other recombinations and gene conversions (Lindegren, 1953; Mitchell, 1955; De Serres, 1956) could occur by interaction or exchanges among the side chains of DNA.

SUMMARY

DNA synthesis is limited to interphase and frequently to a part of the

interphase stage. When only a part of interphase is involved, DNA synthesis may occur at the beginning of interphase, near the middle of interphase or at the end, depending on the tissue and the species. DNA synthesis and chromosome replication occur simultaneously and probably are part of the same sequence of events. Chromosomes become double to radiation breakage as the DNA is synthesized and the basic proteins of the nucleus increase at the same time. RNA synthesis is hardly detectable in the cell during periods of DNA synthesis and also stops or decreases markedly during late stages of division when the chromosomes are condensed. Although this may indicate direct control of the chromosomes in the synthesis of RNA, other interpretations are possible.

By utilizing tritium-labeled thymidine which is a selective label for DNA, and therefore the chromosomes, the distribution of original and new DNA can be determined in individual chromosomes by autoradiography. Experiments showed that the original and new DNA are distinct entities, the integrity of which is conserved during replication. The new DNA is equally distributed to the two daughter chromosomes and it must be assumed that the original DNA is likewise equally distributed. When these chromosomes which have been labeled by one replication in the presence of labeled precursor, are allowed to replicate once in the absence of the label, each regularly produces one labeled daughter chromosome and one completely free of the isotope, except for an occasional sister strand exchange. This behavior supports the idea that replication of DNA follows the scheme proposed by Watson and Crick. Several models of chromosome structure are considered in the light of the experimental findings. A model composed of DNA double helices attached as side chains to a double central axis which separates during replication would explain the behavior of DNA during replication and yet would account for the morphological and mechanical properties of the chromosomes.

Since the chromosomes duplicate before zygotene pairing occurs, crossing over in higher organisms probably in not a part of the replicating process. This rules out the Belling hypothesis which likewise is not supported by the mode of replication of chromosomes. Since one unit of DNA in the original chromosome goes to each daughter, crossing over at least requires the breakage of this original unit at each cross-over. However, it may be noted that if the model is accepted crossing over along the axis is the only one that requires breakage. Recombinations between homologous side chains could be of the type which has been proposed for phage.

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TELOCENTRIC CHROMOSOMES

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INTRODUCTION

Nawashin (1916) expressed the opinion that all centromeres are interstitial. When telocentric chromosomes arise through the misdivision of centromeres in metacentric chromosomes they are either converted into isochromosomes or are lost. This has led to the conclusion that all telocentric chromosomes are unstable and hence do not survive in nature (Darlington, 1939; White, 1954). Nevertheless chromosomes do occur in which centromeres appear to be terminal but it is questionable whether they are truly telocentric and the term "acrocentric" has been used by White (1954) to describe them. Sometimes these chromosomes have structures which can be interpreted as small arms (White, 1954) but, alternatively, these structures may be regarded as parts of a terminal centromere (Cleveland, 1949; Ellerstrom and Tjio, 1950; Love, 1943; Melander, 1950; Melander and Knudson, 1953; Tjio and Levan, 1954). Evidence can be adduced for the occurrence of stable telocentric chromosomes and they raise the following questions:–

(a) Why are some telocentric chromosomes stable whereas others are not?
(b) Since stable telocentric chromosomes exist why are they rare in nature?
(c) Are acrocentric chromosomes actually telocentric?

HYPOTHETICAL STRUCTURE OF TERMINAL CENTROMERES

It must be remembered that the term "telocentric" refers only to the position of the centromere and in no way describes its structure. This may be expected to vary in terminal centromeres which have resulted from the misdivision or breakage of interstitial ones.

Lima de Faria (1949) has examined the centromere of Secale at the pachytene stage of meiosis. He distinguishes three regions within it: an exterior faintly staining region, a chromomere region and an internal region which may or may not contain chromomeres (Lima de Faria, 1956). These three regions are duplicated so that the centromere may be described structurally as a "double repeat" (Lima de Faria, 1949). These regions are a constant feature of all the centromeres that were examined. Similar descriptions have been given in other material by several authors (listed in Lima de Faria, 1956). Lima de Faria considers that the telocentric chromomeres are already divided before metaphase and so are not likely to control the actual division of the centromere or its separation into two daughter centromeres. He believes that the centromeric chromomeres are those

parts of the centromere that are concerned with its movements on the spindle. From observations on interphase and second metaphase chromosomes in Agapanthus and C- mitotic and untreated metaphase in root-tip mitosis in Allium and Galtonia, Lima de Faria (1953) suggests that, in these materials at least, a special region controlling division is located not in the centromere itself but in the most proximal parts of the chromosome arms. Wilson (1956) has arrived at the same conclusion from his observations on *Endymnion non scriptus* and *Scilla sibirica.* Furthermore he provides evidence suggesting that the proximal regions are divided although not separated until the end of metaphase. These regions are included within the centromere as the term is used here, that is, a specialised part of the chromosome with a division cycle of its own and concerned with chromosome movement in the spindle—the "localised kinetochore" of Lima de Faria (1949). Östergren (1951) believes from studiés on centromere orientation that the centromeric chromomeres are divisible lengthways into kinetic and akinetic halves, the former being toward the outer surface of the centromere. From these various observations it is possible to construct a diagramatic representation of the centromere. This is shown in Figure 1.

The consequences of transverse and oblique breaks at certain positions in this structure are shown in Figure 2.

Four points of breakage are considered of which three (Fig. 2, *2, 3, 4*) are breaks within the centromere and the fourth (Fig. 2, *1*) is a break in the arm adjacent to it. Three kinds of telocentric chromosomes will be formed and they can be distinguished by differences in the structure of their terminal centromeres (Fig. 2, types *A, B* and *C*). A telocentric of type *A* has a centromere identical in structure with that of a metacentric chromosome. The centromere of type *B* lacks a proximal region and that of type *C* is only half the centromere of a metacentric chromosome.

Darlington (1939) considered the centromere to consist of a number of

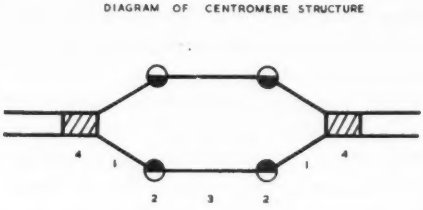

FIGURE 1

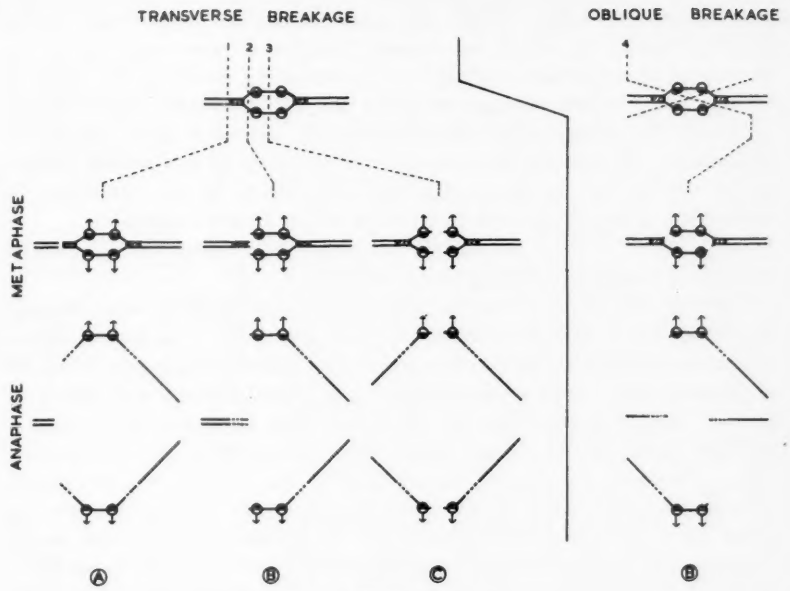

FIGURE 2
(Explanation in Text)

"centrogenes." These were believed to be equally distributed during normal division of the centromere but unequally so with misdivision. A terminal centromere with a high number of centrogenes was considered efficient while one with a low number was thought to be inefficient. With the present model, a dosage effect could operate through differences in number of centromeric chromomeres since telocentric chromosomes of Types *A* and *B* have two centromeric chromomeres and those of type *C* have one. Examples of telocentric chromosomes which may possibly belong to the above categories have been given by Lima de Faria (1956). However, until the structure of a normal centromere can be definitely correlated with its behavior and the structures of abnormal centromeres determined, it is pointless to speculate on the dosage effect of centromeric chromomeres on the behavior of terminal centromeres. The main point that can be made from considering such a hypothetical scheme as that put forward is that chromosomes with complete terminal centromeres can be expected as a result of breakage.

CLASSIFICATION OF TELOCENTRIC CHROMOSOMES

In Figure 3 telocentric chromosomes are classified according to their origins and stability.

Vaarama (1954) suggests that primitive chromosomes had terminal centromeres and here the term "prototelocentric" is used for them. Its application is limited owing to the impossibility in most cases of deciding whether a telocentric chromosome is primitive or recent. Probably the telocentric chromosomes of Holomastigotoides (Cleveland, 1949) and *Ulophysema*

oresundense (Melander, 1950) are prototelocentrics since they exhibit primitive characters and do not appear to be derived from any pre-existing two armed chromosomes. Whether the telocentric chromosomes found in evolutionarily advanced organisms like the bull (Melander and Knudson, 1953) and the mouse (Tjio and Levan, 1954) belong to this category is conjectural. In contrast to prototelocentrics are those telocentric chromosomes which have arisen through the misdivision of the centromere in metacentric chromosomes and they may be either stable or unstable.

(a) *Unstable derived telocentric chromosomes.* Examples of unstable telocentric chromosomes are given in table 1.

Wherever they occur the material proves to be abnormal in other respects —see table 1. The commonest abnormalities of the telocentric chromosomes are non-disjunction and misdivision. These only occur when the telocentric chromosomes are univalent. In wheat ordinary univalents are just as likely to misdivide as telocentric ones (Sears, 1952). Clearly therefore instability is not related to centromere position and it would seem that a terminal position itself has no effect on the behavior of a centromere.

(b) *Stable derived telocentric chromosomes. Nothoscordum fragrans* was found by Koeperich (1930) to have 2n = 16 chromosomes all with median centromeres. Andersen (1931) and Beal (1932) found 2n = 18 chromosomes in *N. bivalve* of which 16 had median centromeres and two chromosomes had terminal centromeres. The same situation was found by Levan (1938)

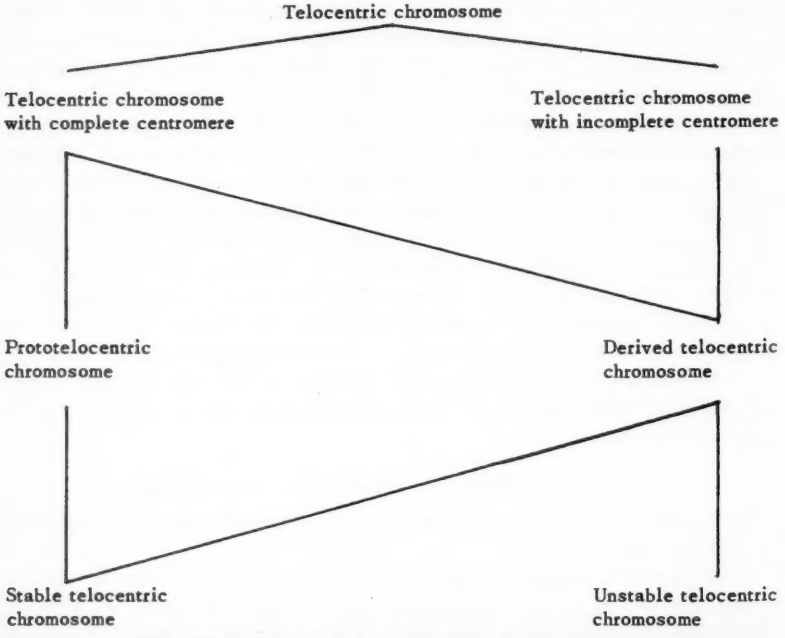

FIGURE 3. Classification of telocentric chromosomes.

TABLE 1

Material	Kind of instability	Conditions for stability	Remarks	References
Campanula persicifolia	Failure of congression Centromere orientated away from spindle			Darlington and La Cour (1950)
Fritillaria kamtschatkensis	Non-disjunction at pollen grain mitosis		Plant asynaptic; 98 per cent of univalents misdivide	Darlington (1939)
Zea mays	Non-congression at metaphase Isochromosomes produced through non-disjunction	When paired at meiosis	Plant trisomic. Extra chromosome telocentric	Rhoades (1940)
Triticum vulgare L.	Misdivision and non-disjunction at meiosis when unpaired	When paired at meiosis	43 chromosome speltoid plant Telocentric chromosome pairs with normal homologue to give heteromorphic bivalent	Huskins and Spier (1933)
Triticum aestivum L. en Thell	Misdivision and non-disjunction at meiosis when unpaired; giving isochromosomes	When paired at meiosis	Plant monosomic	Sears (1952)

in a strain of *N. fragrans*. Levan and Emsweller (1938) found another strain of *N. fragrans* with 2n = 19 chromosomes consisting of 13 chromosomes with median centromeres and six chromosomes with terminal centromeres. Each of the six telocentric chromosomes is described as having a small "proximal satellite." However, Sato (1942), studying a similar strain of *N. fragrans*, claimed that only four of the telocentric chromosomes had satellites. All these authors agree that the telocentric chromosomes have resulted from the breakage of chromosomes with median centromeres but do not state whether they consider breakage to have occurred at the centromeres or in regions adjacent to them. However, Kurita (1953), re-investigating the same material, believes that breakage was through each centromere and considers the "proximal satellites" of each telocentric chromosome to be visual parts of the terminal centromeres. Levan (1935) describes telocentric chromosomes in the karyotypes of *Allium pendulinum* Ten. 2n = 18 and *Allium zebdanense* Boiss et Noe. and considers them to be derived from chromosomes with median centromeres. Sato (1942) describes *Allium condensatum* 2n = 17 as having two telocentric chromosomes in its complement and he suggests that this species may have originated by breakage at the centromere of one of the median chromosomes of a species like *Allium obligonum* with 16 chromosomes having median centromeres. Cave and Bradley (1943) described two kinds of plants of *Miersia chilensis*, one kind with 2n = 20 and the other 2n = 21 chromosomes. All the chromosomes of the 2n = 20 plant have median centromeres and they pair regularly at meiosis to give ten bivalents. The karyotype of the 2n = 21 plant consists of 19 chromosomes with median centromeres and two rod-shaped chromosomes with terminal centromeres. At meiosis nine bivalents and one trivalent are regularly found. The trivalent results from the pairing of the two telocentric chromosomes with a chromosome having a median centromere. It is thought that the telocentric chromosome arose by breakage adjacent to the centromere in one of the chromosomes with median centromeres. If this interpretation is correct then breaks must have occurred on each side of the centromere of this chromosome, either simultaneously or separately depending upon the time of occurrence. Orientation of the trivalent at metaphase was always such that at anaphase both telocentric chromosomes went to one pole and the metacentric chromosome to the other. A similar association of two telocentric and one metacentric chromosomes was also found in Campanula (Darlington and La Cour, 1950). The Y and two X chromosomes in *Rumex acetosa* (Kihara and Ono, 1925) and *Humulus japonica* (Kihara and Mirayoshi, 1932) pair to give trivalent configurations similar to those found in Miersia and Campanula. This suggests that the two X chromosomes in each of these species is telocentric and that they are the arms of what was once a single metacentric chromosome. Love (1943) found a pair of telocentric chromosomes in a white chaff mutant of Dawson's Golden Chaff Wheat. These telocentric chromosomes were homologous and paired regularly at meiosis. The mutant plant when crossed with a normal plant gave plants with one telocentric

chromosome which paired at meiosis with one arm of a normal two-armed chromosome to give a heteromorphic bivalent. This indicates that the telocentric chromosome had arisen by breakage at or adjacent to the centromere of a metacentric chromosome. In *Oxalis dispar* 2n = 12 the karyotype consists of two long chromosomes with submedian centromeres, three median chromosomes with sub-terminal centromeres and seven medium chromosomes with terminal centromeres (Marks, 1956). At meiosis one of the telocentric chromosomes pairs with one of the sub-terminal chromosomes to give a heteromorphic bivalent suggesting as in the case of Wheat (Love, 1943) that one telocentric chromosome and possibly the others arose as the result of breakages at or near the centromere in metacentric chromosomes (Marks, 1957). Ellerström and Tjio (1950) describe a pair of homologous telocentric chromosomes in the karyotype of *Phleum echinatum*. They consider that the centromeres of these telocentric chromosomes are structurally equivalent to those of the metacentric chromosomes. In one plant of *Campanula persicifolia* Darlington and La Cour (1950) found a naturally occurring stable telocentric chromosome as well as in other plants unstable telocentric chromosomes which arose by misdivision of metacentric chromosomes during the course of the investigation.

In animals there is also evidence for the existence of stable telocentric chromosomes. Cleveland (1949) considers the chromosomes of Holomastigotoides to have terminal centromeres while those of *Ulophysema oresundense* are undoubtedly telocentric (Melander, 1950). All the chromosomes in the karyotypes of the bull are probably telocentric (Melander and Knudson, 1953). Sachs (1953) believes the Y chromosome of *Microtus agrestis* to be telocentric while Tjio and Levan (1954) give evidence to show that all the chromosomes but two in the karyotype of the mouse have terminal centromeres.

RARITY OF NATURALLY OCCURRING STABLE TELOCENTRIC CHROMOSOMES

The types of gametes which occur with *C* telocentrics owing to the misdivision of one univalent are shown in Figure 4.

They are of two kinds. The first are deficient in a chromosome arm and may be expected to be at a disadvantage in competition with normal gametes unless the lost arm is genetically inert or its loss is nullified by polyploidy. The second have the telocentric chromosomes in excess of the normal or abnormal complement. If these gametes compete successfully with normal gametes they will give polysomic individuals which are selectively disadvantageous in sexually reproducing organisms and will be eliminated (Dobzhansky, 1951). Gametes containing telocentric chromosomes of types *A* and *B* will also be abnormal. A gamete containing two telocentric chromosomes each consisting of one complete arm of what was once a metacentric chromosome could only arise by the simultaneous misdivision of two homologous univalents with subsequent sorting out of the appropriate chromosome arms. Individuals arising from the fusion of

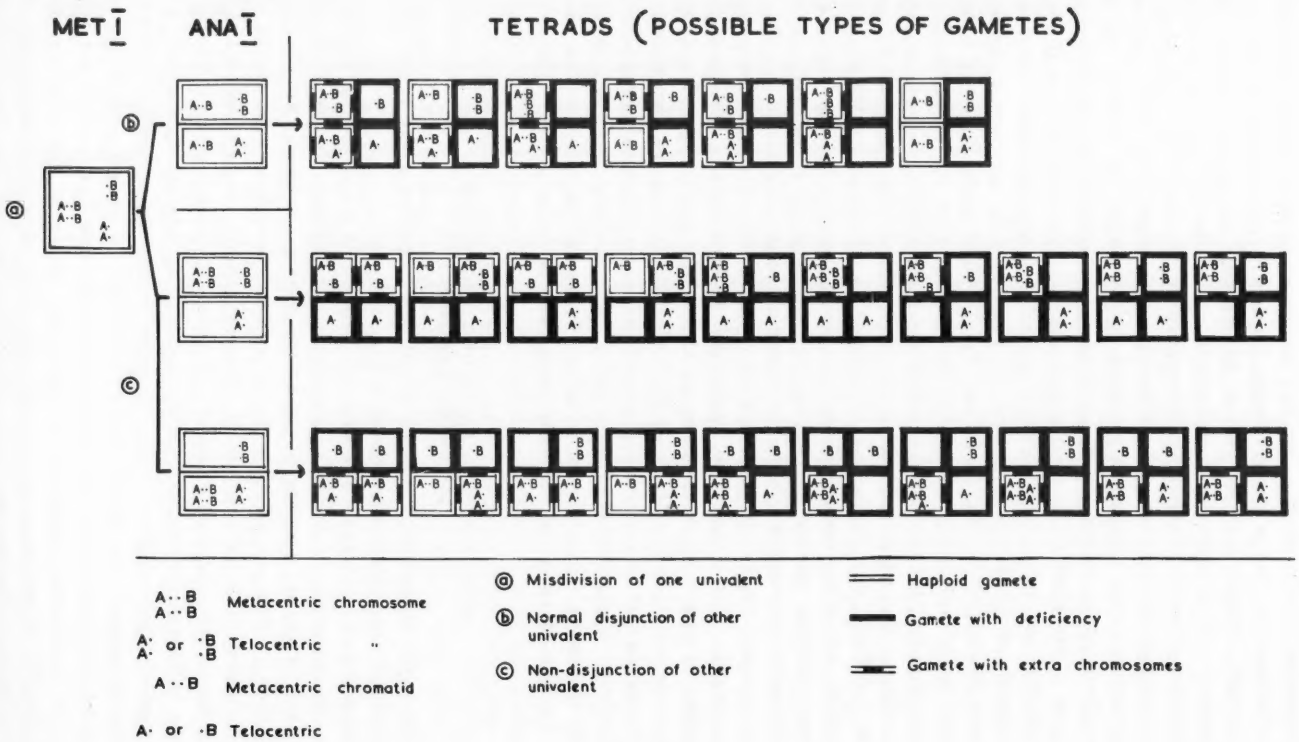
MET I
ANA I
TETRADS (POSSIBLE TYPES OF GAMETES)
a
b
c
A··B Metacentric chromosome
A··B
A· or ·B Telocentric "
A·
A··B Metacentric chromatid
A· or ·B Telocentric
a Misdivision of one univalent
b Normal disjunction of other univalent
c Non-disjunction of other univalent
Haploid gamete
Gamete with deficiency
Gamete with extra chromosomes

FIGURE 4

two such gametes would be genetically balanced but are likely to be extremely rare.

Derived telocentric chromosomes can be perfectly stable and yet they are rare. They are so not for any reason connected with centromere inefficiency but because the gametes containing them are usually genetically unbalanced and die. Indeed where they do occur it follows that special genetical conditions may be responsible for their survival. This is so in *Oxalis dispar* in which the short arms of sub-terminal chromosomes are evidently deleterious. As a result derived telocentric chromosomes consisting of long arms only have a selective advantage over the original sub-terminal chromosomes (Marks, 1957).

ACROCENTRIC CHROMOSOMES

If the terminal structures sometimes found in acrocentric chromosomes are in fact short arms, it follows that these chromosomes will have centromeres identical to those of metacentric chromosomes. It is therefore the structural normality of their centromeres that accounts for the stability of such chromosomes.

Since there is evidence to show that a terminal centromere is not inefficient it follows that the presence of a minute second arm is immaterial from the point of view of centromere efficiency. Consequently the alternative suggestion that the terminal structures are part of the centromere is perfectly reasonable. Since it is not always possible to demonstrate terminal structures it follows that the term "acrocentric" is sometimes unnecessary as chromosomes without terminal structures are quite likely to be truly telocentric.

SUMMARY

(1) The effects of breakage in four positions upon a centromere such as that described by Lima de Faria are considered. Three kinds of telocentric chromosomes may be expected to arise and one of them will have a centromere similar to that of a metacentric chromosome.

(2) Examples of telocentric chromosomes are listed and classified. There is no evidence that a telocentric chromosome is unstable because its centromere is terminal.

(3) It is suggested that telocentric chromosomes are rare not because their centromeres are inefficient but because they have a low chance of survival. Many of the cells in which they occur are likely to die because of genetical unbalance brought about by chromosome deficiencies or excesses.

(4) It is suggested that the term "acrocentric" may be sometimes unnecessary.

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THE TEMPERATURE SENSITIVITY OF SOME AMERICAN FRESHWATER FISHES

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INTRODUCTION

Temperature is an important environmental variable; it affects metabolism directly and it influences other factors in the environment. It has greater bearing on poikilothermous animals than on those that can regulate their internal body temperature. There is a vast array of investigations of the effects of temperature on fishes, but few of them deal with thermal sensory mechanisms as such though these are the key links which permit the animal to gauge changes in environmental heat flow before adjusting to them.

Investigations on the temperature sense of fish are essentially of two kinds: behavioral and neurophysiological. Among the former, we may distinguish between conditioned reflex experiments and those in which temperature preferences were observed.

Temperature preference experiments have been further subdivided (Sullivan, 1954) into: 1. observation of activity as fish encounter or are subjected to rapidly changing temperatures. 2. experiments in which an activity is observed at different temperatures after the fish have become equilibrated to them, or, 3. experiments in which fish are thoroughly acclimated to a given temperature before observations of activity are made at that temperature.

In most cases, temperature preference experiments do not seek to explain the physiological mechanisms by which the responses come about, but they do show the existence of a temperature sense.

For example, in early experiments on temperature preferences in fish, Wells (1914) concluded that fish could detect exceedingly small (0.1°C.; 0.18°F.) variations in the temperature of the surrounding water and must, therefore, possess a very delicate "temperature regulating mechanism." This and other early papers were subject to the criticism that convection currents rather than temperature as such may have been the stimuli responsible for the observed behavior, but this source of bias has been eliminated in most recent studies.

In temperature preference experiments of type 1 (above), Sullivan and

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Fisher (1953) have shown that in a horizontal gradient, young brook trout (*Salvelinus fontinalis*) "select" a certain temperature. Likewise temperature selection by salmonids in a vertical gradient has been shown by Graham (1949) and Brett (1952).

In temperature preference experiments of the second type, Sullivan and Fisher (see Sullivan, loc. cit.) have shown that maximum cruising speed, as measured at different equilibration temperatures, is greatest at the selected temperature.

Experiments of type 3 (above) indicate that there is an increase in the maximum cruising speed of which goldfish and trout are capable at each of a number of acclimation temperatures as the acclimation temperature rises to a peak corresponding approximately to the "final preferendum temperature", (that is, the temperature at which the selected and acclimation temperatures coincide), Fry and Hart (1948), Graham (1949).

With regard to conditioned reflex experiments, Bull (1936) demonstrated temperature discrimination of +.03 to .07°C. (.06 to .13°F.) in several species of marine teleosts. Dijkgraaf (1940) also working with conditioned reflexes used an experimental procedure which did not permit as fine a discrimination as Bull's (loc. cit.), but he showed, beyond doubt, that it was the nerve endings in the skin and the spinal nerves which acted as receptors and conductors of temperature sensations. He also demonstrated the ability of fish to discriminate between a rise and a fall in environmental temperatures. This has some bearing on our experiments and will be discussed more fully later. That this acute perception of changes of the heat energy flow in the environment is due to areal or spatial summation of impulses has been intimated by Dijkgraaf (1940) and recently been demonstrated by Bardach (1956).

Among the neurophysiological studies, the investigations of Hoagland (1933, 1935) and his collaborators are of interest. He tapped lateral line nerves of a number of fishes and registered the reaction of the lateral line receptors to temperature changes. It was later shown by Dijkgraaf (1940) that these organs were not primarily concerned in temperature reception. Sand (1938) and Hensel (1955) showed that a part of the lateral line system, namely the Ampullae of Lorenzeni in elasmobranchs, are very sensitive to temperature changes. Skin-nerve preparations either isolated or on the living animal have as yet not been investigated in teleost fishes.

Even though acute temperature sensitivity of fish has been demonstrated in the past, it appeared worthwhile to make some observations on American freshwater fishes. It could again be demonstrated that the level of discrimination for warming or cooling lies somewhere around .05°C. (.09°F.), possibly even less. Thus, we extended and amplified the observations of Dijkgraaf and Bull.

MATERIALS AND METHODS

The experimental animals were 1. goldfish (*Carassius auratus*). Though this is not strictly an American freshwater species, it is by now widespread in American waters and readily adapts itself to laboratory conditions. It served well for the elaboration of our techniques. 2. yellow bull-

head (*Ictalurus natalis*) 3. creek chub (*Semotilus atromaculatus*) 4. pumpkinseed (*Lepomis gibbosus*) 5. rainbow trout (*Salmo gairdneri*). Thus, four soft-rayed and one spiny-rayed species were tested for their acuity of temperature perception. The goldfish occurs naturally in standing water and the creek chub in running water environments, while the bullhead is a bottom dwelling form and the trout a cold water representative. It is felt that this constitutes a fair representation of the American freshwater fauna.

In most experiments, six fish were tested simultaneously. In others, those on cooling, only three or four fish were used. The fish were between 6 and 10 cm. total length, except for the goldfish in the warming experiments which were 10–15 cm. long. All of the goldfish used in the cooling experiments were sexually mature. All other fish were either immature or possessed regressed gonads.

Six glass aquaria of 9.5 liters capacity measuring 19 × 19 × 27 cm. were placed behind permanent wood and beaver-board screens, (Figure 1). On their long sides they were painted with black asphaltum paint so that the fish could not see each other. An automatically controlled fluorescent

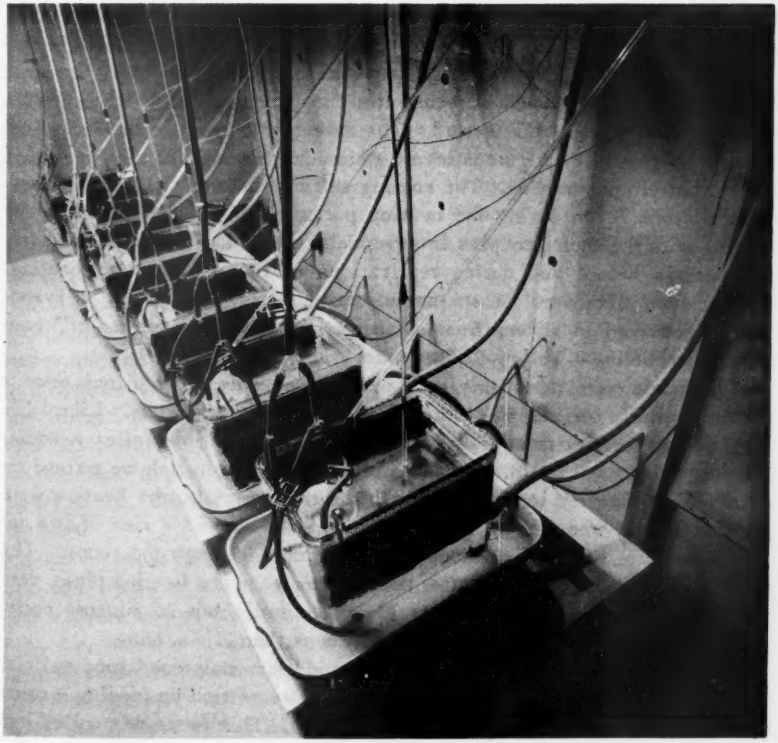

FIGURE 1. View of experimental arrangement for warming trials, looking towards screen.

lamp illuminated the aquaria for fourteen hours each day. The observer could watch each fish individually as well as two to three aquaria at one time from his position, in the dark through peepholes in the screen. Glass tubes reached into the aquaria from the observer's galley so that food could be distributed through these to each individual fish. Copper-constantan thermocouples (30 B and S gauge) were placed in the aquaria and connected through a multiple switch to a Leeds Northrup Speedomax recording potentiometer. An air-lift filter with glass wool, and charcoal was placed in each aquarium. The tanks stood in shallow pans on glass supports in a water bath about 4 cm. deep. Under the pans, aluminum plates were placed on 115 V. 500 watt "Chromalox" strip heating units (Wiegand) connected to plugs on the outside of the partition so that each aquarium could be heated individually from the outside. Lucite supported screens of plastic cloth were placed in the aquaria, 2.5 cm. from the bottom to prevent the fish from touching the glass bottom and thus receiving a heat cue from the glass rather than the surrounding water. Thus, the fish were left essentially undisturbed throughout a series of experiments.

In later experiments with a deep water bath, some trials were made lining all aquarium walls with fiberglass screening so that the fish could nowhere touch the wall. When shallow water baths were used, only the bottom and the first 2.5 cm. of the sides were barred by fiberglass screens. In the center of the plastic screens, airstones were placed which emitted a violent stream of bubbles upward. The extent of stirring in the individual aquaria was kept at a vigorous level to eliminate changes of the convection current pattern on warming. The stirring sufficed to keep the water temperature uniform, even during the heating period. This was ascertained by probing with the thermocouples in several locations in all aquaria for varying periods before and during replications of the experimental heating. Stirring even eliminated the sub-surface temperature gradient due to evaporation, observed in an unstirred aquarium with the "optical tester" (see section on cooling—Yellow Bullhead).

All animals were thoroughly acclimated to laboratory conditions prior to the ten training periods which preceded the experimental runs. Like Dijkgraaf we found that most fish would learn to form simple reflex associations in even fewer trials. Only four of the three dozen fish we trained required more than ten trial periods. In the training runs, the heaters were plugged in and when the recording galvanometer showed a rise of two degrees in water temperature, food was introduced through the tubes. The first trace of a rise occurred after ten minutes when the heating plugs were disconnected. Warming the water 2°C. required about 20 minutes corresponding to an average change of slightly less than .1°C./min.

Initially, we used the super-natant liquid from moistened food pellets, then the food pellets themselves, and finally we settled on feeding a moist mixture of cat-food enriched with cod liver oil. This paste was rolled into small pellets between the fingers and introduced through the tube. It rolled down and fell in the same corner every time. Depending on the species,

the food was either seen, or smelled and tasted. Each species developed a characteristic behavior after forming the heat rise-food association. This is described in a later section. From the tenth trial on, the sequence of warming of our six fish was determined from a table of random numbers and one fish was chosen as control by the same means. On the control fish, a sham operation was performed differing from the test only in inserting and immediately withdrawing the heater plug. Only one trial was performed each day and the control fish was not fed.

It was argued that lack of response in the control compared to the other fishes in the series should demonstrate their reactions to changes in temperature. Furthermore, since a characteristic behavior pattern developed, the observers could ascertain the lowest temperature increase at which the response of an individual fish occurred. The tests were performed at widely varying times of the day and occasionally an entire day was skipped so that no rhythm of food expectancy might develop. We took turns in making the observations on alternate days.

Our recorder permitted the distinction of ± .1°C. with certainty and the interpolation to .05°C. with reasonable accuracy.

There remained the possibility that the fish reacted to an extraneous cue, associated with the performance of the test rather than to a temperature rise itself. The following extraneous cues were considered:

1. Sound. Continuous observation of the experimental animals showed that they did not react to the click of the selector switch or the plugging in of the heater. The observer's gangway was padded with a rubber mat for shock and soundproofing and no reaction to footsteps was noticed. Furthermore, a filter pump serving an aquarium above our laboratory provided, to our ears at least, a constant background noise which masked slight disturbances.

2. A small electric shock might be administered to the fish when the plug was inserted due to conduction over the moist surfaces of the heating elements and the aquarium wall into the water itself. No consistent evidence of a reaction to such a cue was observed.

3. Vibration due to heating of the understructure beneath the aquarium (the metal plates and the water pan) might alert the fish regularly in such a manner that it would respond to this, rather than to the heat rise, especially if the vibration coincided with a certain level of warming in the water bath.

Such a cue, if not ruled out, was at least proved unlikely in the following manner: after the warming experiments were terminated, the experimental arrangement was altered for cooling tests. A large, glass tank of 91.5 × 35 × 31 cm. was fitted with two inflow hoses from the mixing faucet in the room and one outflow to form a water bath around the experimental aquaria. Its temperature, as well as that of the aquaria therein, was measured as outlined and the observer still looked through the peepholes. Before proceeding with the cooling experiments, we warmed three of the pumpkinseeds trained in the first experimental arrangement, by flushing the water bath

with warm water. One other fish was kept as a permanent control outside the bath.

Under this different experimental arrangement where warming of the water was, again, the only cue, the fish reacted exactly as in the previous experiments and at the same levels of heat rise. This, in conjunction with experimental results from the literature, strongly suggested that our fish were indeed reacting to temperature changes and nothing else.

The cooling experiments which were performed on rainbow trout and on goldfish were conducted in the modified experimental arrangement described above. Three, later two, experimental fish were observed before and during cooling and compared to one control, which was not cooled. Two experimental fish proved the most that an experimenter could watch simultaneously. This was necessary since temperature changes in the water bath showed only a short lag from the inflow end to the center and the outflow at the other end.

The experiments, conducted between January 1955 and May 1956, were discontinued for a period of five weeks during the summer of 1955, because of excessively high room temperatures and bacterial contamination of tap water used up to that time. Thereafter, the aquaria were kept filled with pond water. The temperature in the row of six aquaria differed at the outset of the experiments by, at most .7°C.; however, each aquarium maintained its base temperature without variation during the period from the onset of heating to the recorded trace of a heat rise. The water temperatures at the beginning of a day's trial were between 16°C. in January, and 22°C. in June, with slow, small variations due to day to day changes in outside temperature.

Statistical tests of our results are discussed in a separate section and are summarized in tables III to V.

RESULTS

Reactions to Warming. Goldfish. (table I.) The positive reaction of this species was a raising of the dorsal fin, if it was not previously taut. This was immediately followed by a feeding "dance" in which the animal assumed a position about 45° from the horizontal while searching for food on the plastic screen. Finally, the animal moved to the location where the food pellets dropped down.

In the most decisive tests the fish were at rest on the plastic screen or stationary in mid-water and their reactions were sharply defined. On a few other occasions, the fish were not quiet at the beginning of the experiment or alerted by an extraneous cue; then the trial run was discarded regardless whether the fish was a control or not. At times, the control fish were somewhat restless but not sufficiently so to invalidate their performance; they were therefore noted as C –.

Tabulation of the trial runs in the warming experiments of goldfish as well as the other species showed that:

TABLE 1

SUMMARY OF WARMING EXPERIMENTAL TRIALS

Trial Number	Goldfish			Yellow Bullheads			Creek Chubs			Pumpkinseeds		
	Number of Positive Responses	Mean Level of Response (°C.)	Number of Responses <0.1°C.	Number of Positive Responses	Mean Level of Response (°C.)	Number of Responses <0.1°C.	Number of Positive Responses	Mean Level of Response (°C.)	Number of Responses <0.1°C.	Number of Positive Responses	Mean Level of Response (°C.)	Number of Responses <0.1°C.
11	4/5	.34	1/4	3/5	.43	0/3	5/5	.83	0/5	4/5	.11	3/4
12	1/5	.10	0/1	4/5	.16	3/4	5/5	.57	2/5	4/5	.11	2/4
13	5/5	.35	1/5	2/5	.23	0/2	5/5	.24	0/5	4/5	.09	2/4
14	4/5	.31	1/4	4/5	.49	1/4	3/5	.05	3/3	5/5	.08	2/5
15	4/5	.29	2/4	3/5	.26	0/3	3/5	.22	0/3	4/5	.08	2/4
16	4/5	.24	0/4	5/5	.08	4/5	5/5	.30	1/5	3/4*	.20	1/3
17	3/5	.08	2/3	4/5	.22	0/4	3/5	.23	0/3	4/4	.05	4/4
18	5/5	.07	4/5	4/5	.06	3/4	3/5	.12	1/3	4/4	.08	2/4
19	4/5	.20	0/4	4/5	.08	3/4	3/5	.27	0/3	2/4	.13	1/2
20	4/5	.06	3/4	3/5	.05	3/3	4/5	.19	0/4	3/4	.20	2/3
21	4/5	.22	2/4	2/5	.13	1/2	4/5	.20	0/4	4/4	.08	2/4
22	3/5	.23	0/3	2/5	.08	1/2	4/5	.18	1/4	2/4	.10	0/2
23	4/5	.22	1/4	4/5	.21	1/4	4/5	.16	1/4	3/4	.06	2/3
24	4/5	.31	1/4	2/5	.10	0/2	4/5	.08	2/4	4/4	.05	4/4
25	4/5	.10	1/4	3/5	.10	1/3	5/5	.12	1/5	3/4	.06	2/3
Totals	57/75		19/57	49/75		21/49	60/75		12/60	53/65		31/53
Mean of Means		.21°C.			.18°C.			.25°C.			.10°C.	
Controls												
Discarded	0			3			1			2	*One fish died	
"C-"	6			1			3			3		

1. The lowest level of temperature change observable by us was reacted to in the earlier as well as the later trials (see table I: Numger of Responses below 0.1°C.). Once a change in heat flow had become meaningful through association, very small changes elicited the learned reponse.

2. The heat rise during training was +2.0°C. Most fish reacted to this by raising their dorsal fins followed by cruising through the aquarium. In all species some individuals reacted more violently than others. The speed of learning seemed to depend both on individual variability in general alertness and on the rest position of the fish with respect to the feeding tube. If they were close to the tube when they were first fed they often associated the heat rise with food after two or three trials. The variability of response during the training trials was such that one or two fish already reacted to a heat rise of .2°C. after training trial 3 or 4; one or two others, on the other hand, still had not formed the association, even to a heat rise of 2°C. after the 6th or 7th training trial.

In spite of these variations one could observe an overall increase in the acuity of temperature perception during the learning trials (not included in tables). Thus, for instance, three goldfish out of five reacted to heating in training trial 8 at a mean of .71°C. while the remaining two fish did not show a clear-cut reaction. The mean of four out of five bullheads in training trial 6 was .92°C. but .43°C. in trial 11, the first experimental trial (table 1).

3. Observations at other than test period showed that using the fish as experimental and control animals resulted toward the end of the trials in an increase of spontaneous activity as if the fish were finally alerted to look for food frequently and at random. In the bullheads, for instance, this was reflected in some increase of runs which had to be discarded (table 1: number of positive responses in trials 21–25 as compared to trials 16–20).

It has been mentioned that some fish learned faster than others. If one followed the performance of any one fish through the learning and experimental trials it appeared that those which had learned early and well also, by and large, performed positively during the trials more frequently than the ones which had been slower to learn. Creek chub No. 2 had learned well after training trial No. 4. This same fish showed 20 positive performances in the 21 trails conducted after the warm-food association was definitely established. At the other extreme, fish No. 6, in the same group did not make a positive response until trial 10 and gave only 7 positive performances out of the 15 trials conducted after learning was established. Creek chub No. 6 had the poorest record of any individual we trained regardless of species.

Because the examples just cited are extreme cases and ordinarily the variability in learning was much less between individuals of the same species, we are of the opinion that such variation represents individuals differences in alertness and "intelligence" rather than differences in the threshold of temperature sensitivity.

This may also serve to explain any decrease in the number of responses

below .1°C. toward the end of the 75 trials; the animals were restless and the exact onset of the reaction was difficult to ascertain. The temperature in the aquaria also fluctuated slightly from day to day and it may be that some of these changes were rapid enough to be confused with trial warming.

4. The number of total positive runs is significantly larger than could be accounted for by chance: 57 positive performances in 75 trials were recorded for the goldfish (See statistical treatment section). A positive experimental run was recorded when trained fish reacted decisively, in the manner described, upon a heat rise between .05°C. and 1.0°C.

5. The lowest temperature rise at which we could observe a clear-cut reaction was .05°C. with a mean of .21°C. for 57 positive trials.

6. At the onset of the experiment, the position of the fish in the aquaria was often identical from day to day. This was interpreted as being the place with the least disturbance by the air driven circulation. Random exchange of fish from one aquarium to the other for trials 18 through 25 brought no change in reaction. The typical location of a fish in an aquarium then turned out to be a trait of the fish rather than the aquarium, inasmuch as the fish in the new aquarium occupied the same position it held in the old, more often than not. This suggested that the current pattern in the six aquaria was relatively similar.

Yellow bullheads. (table I.) This species, at the beginning of a trial, would frequently hang upright along the sides of the aquarium as if they were asleep or resting. As heating was perceived, the barbels first moved and then stiffened, followed immediately by excited undulatory swimming movements to a place near the feeding tube where the food pellet was scooped up. Here again the behavior became harder to interpret as the animals proceeded with their experimental runs because they became more alert and easily excited. The lowest temperature responded to was around .05°C. with a mean of .18°C. in 49 positive trials.

Creek chubs. (table I.) These are sight feeders and their characteristic behavior after training consisted in a rapid dart into the vicinity of the feeding tube. This was frequently followed by an actual charging of the tube. At times this charge was so vigorous that, much to the annoyance of the observer, fish in other aquaria were alarmed and frightened by this noise and dashed about their tanks. Here, as in the two previous species, the earliest response occurred at a rise of around .05°C. This low level of response was reached only 12 times in 75 trials as compared to 31 in the pumpkinseed (65 trials), 21 in the yellow bullhead and 19 in the goldfish. The mean level of response at .25°C., though the highest among the four species, is still similar to at least two of the others. It might be suspected that creek chubs have a less acute temperature sensitivity than the others but the mean combined with the above behavioral observation rather points to the experimenter's insufficiency in ascertaining the exact level at which the fish reacted.

Pumpkinseeds. (table 1.) These small centrarchids are not only the quickest to train, but they were also easiest to observe. In 31 out of 53

positive trials (one fish died after trial 15), they reacted at a heat rise of less than .1°C. so that even the mean does not exceed .1°C. It is possible that acuity of temperature sensitivity differs slightly from species to species. It is more likely, however, that these differences again reflect the failure of observers to detect in the other species the first trace of a positive response to the stimulus. The centrarchids raise their dorsal fin and then dash to the tube, often attacking it like the creek chubs.

Reactions to Cooling. Rainbow trout. (table 2.) Cooling was achieved in the large glass aquarium fitted as a water bath and described above, with observations easily made through both front aquarium walls.

The fish formed the following conditioned reflex to cooling with subsequent feeding: quick raising of the dorsal fin, if not previously taut; one or two rapid dashes around the aquarium, and then a position was taken up opposite the feeding tube. They did not approach the tube closely but stayed between one and three inches away from it. When the food finally dropped, they made a rapid dash and took it before it had touched the screen bottom. The reaction was characteristic and there was no mistaking it for general excitement. After the usual 10 training trials, two fish, in 11 experimental trials each, were compared to the reactions of one control fish which was not cooled. Cooling proceeded at a rate comparable to warming, namely, slightly less than .1°C./minute. In only one out of 22 trials, did the fish not give a clear-cut reaction and one in 11 control observations was somewhat disturbed. We did not observe reactions at less than –0.1° C. and the mean of all 22 trials was .24°C. After the cooling trials were

TABLE 2

SUMMARY OF COOLING EXPERIMENTAL TRIALS

Trial Number	Rainbow Trout (reacted at drop of °C.)			Goldfish (reacted at drop of °C.)		
	Fish No. 1	Fish No. 2	Mean	Fish No. 1	Fish No. 2	Mean
11	.10	.10	.10	.25	Disc.	.25
12	.20	.20	.20	.10	.15	.13
13	.15	.15	.15	.05	.15	.10
14	.25	.20	.23	.10	.10	.10
15	.70	.20	.45	.05	Disc.	.05
16	.10	.30	.20	.15	Disc.	.15
17	.40	.20	.30	.05	.10	.08
18	.25	.40	.33	Disc.	.10	.10
19	Disc.	.30	.30	.20	.15	.18
20	.15	.30	.23	.10	Disc.	.10
21	.20	.10	.15	.05	.05	.05
Mean; °C.	.25	.22		.11	.11	
Mean of Means; °C.			.24			.12
Discarded controls	0			0		
"C-" Controls	1			2		

run, the fish that had been so conditioned were subjected to slight warming before cooling. This was not done more than twice as even the second trial might constitute learning. In both trials there was evidence that the warming was perceived at approximately 0.1 to 0.2°C., but the reaction of the fish was one of becoming alert rather than taking up its typical feeding position. As the water in the bath was changed to cold, the rise was checked for about ten minutes before the cooling began in the experimental aquaria. During this period, frequency and velocity of swimming movements were reduced compared to both the warming and the cooling periods.

Goldfish. (table 2.) Two goldfish were trained to react to a drop in water temperature and compared to one control as described for the trout. One of the fish soon showed the typical "feeding dance" that is, canting and rapidly snapping at the wire screen as on warming, while the other experimental fish, early in the training seemed to perceive that food dropped out of the tube, rather than noticing the food pellet when it was on the bottom. Its positive reaction became a light attack of the tube but never bumping or jarring it. Half way through the trials, the second experimental fish also assumed this reaction.

The last four trials were performed with a plastic wire enclosure in the aquarium in order to eliminate tactile temperature cues from the aquarium walls. These tests were considered important to compare the thresholds for warming and cooling.

Twenty-two trials were run with five discarded observations, but none out of the 11 control observations were inadequate though two were noted as C–. A temperature drop of less than .1°C. at a rate of change of –.08° C. per minute was the smallest change in heat flow reacted to and the mean of the twenty-two trials was .12°C. compared to a mean of .21°C. on warming. This difference in means of warming and cooling trials may, in part, be explained by our increased proficiency in detecting minimal responses as the former tests were made one year before the latter.

After the cooling trials we subjected the goldfish to two trials with slight warming before the customary cooling. Both fish showed their typical feeding reaction at + .1°C., and like the trout, moved less frequently and slower during the transition from warming to cooling. As soon as a drop in temperature appeared on the record, the fish resumed their more violent feeding movements.

These observations suggest that nerve adaptation to a new level of environmental temperature occurs fairly rapidly and that on these two occasions the fish perceived a drop as well as a rise in temperature as changes not qualitatively different from one another. Two trials were probably not sufficient to make the distinction of such small differences meaningful to the animal.

Dijkgraaf (1940) on the other hand, trained Phoxinus to distinguish between a drop and a rise in temperature by feeding the fish on warming and punishing them on cooling. The number of training trials is not given and he did not work with our small temperature differences, but he postulated

that fish can, and do, differentiate between heating and cooling. The question of the physiological mechanisms involved was left open.

Yellow bullhead. Some of the experimental animals of this species were observed through an "optical tester" (kindly loaned by the Ann Arbor Optical Co.). This instrument is capable of rendering visible temperature gradients of less than 0.1°C. and is generally used in testing lenses of mirrors for aberrations. It can be considered to be similar to the Foucault knife edge technique for testing lenses or mirrors. Thus, temperature gradients in the water which are also density gradients are easily observed with this instrument. In this manner, it is possible, for instance, to view the cooling effects of evaporation at the surface of an aquarium. Bullheads were chosen for these experiments because they lie relatively still at the bottom of any experimental tank and cold water can be led on to them by placing a small ice cube on the surface of the water. The cold water which flows down from the ice cube can be followed by means of the "optical tester" and the exact instant when it reaches the fish can be seen.

When the colder water slowly sank onto the dorsal surface of the fish under observation, representing a sharp drop of the temperature there by about .5°C., it reacted instantaneously by a very rapid escape from its customary position. This observation could be repeated several times after letting the fish come to rest between tests. The reaction was always instantaneous; there could be no doubt that some skin receptors in the dorsal region were responsible for the perception of temperature, and perhaps, density changes.

These observations revealed another interesting fact. Even when at rest, but certainly when slightly agitated, the surface evaporation produced sufficient temperature changes in the water so that density gradients corresponding to almost .1°C. were continuously observable. Ours was a small aquarium and in it such density gradients might be very pronounced; even so, the "thermal background noise" in the form of small and rapid fluctuations of temperature in the surroundings of a fish must be considerable.

Yellow bullheads were also trained to cooling in the arrangements described for goldfish and trout. They appeared to learn very quickly, much more rapidly than they succeeded in making the warm-food association.

In their resting position they touched the aquarium walls with their barbels. There the effects of cooling by means of a water bath would be noticeable long before reaching the thermocouple in the middle of the aquarium. Cooling tests with this species were discontinued because of this peculiar "learning behavior" which did not seem to be present, at least with the goldfish; several trials were run where fish of this species were entirely barred from touching the walls and no differences in either time or threshold of response were revealed.

STATISTICAL TREATMENT OF THE DATA

Statistical tests were applied to our data principally to examine the pos-

sibility that there was divergence of opinion by the authors both as to kind and degree of response observed.

Tests of the total number of positive responses on warming (pooled results of both authors, table I) for each species were made using the "two way" X^2 method including Yates' correction as outlined by Bliss and Calhoun (1954).

In making the tests, the "C –" and discarded controls shown in table I were considered "positive" responses. In like manner, discarded indecisive results from experimental fish were included as negative results because we have no *a priori* resaon to do otherwise. As tables 1 and 2 show, decisive results were obtained for both controls and experimentals about three times out of four. The test is thus one of independence between category (that is, control or experimental) and tendency toward positive response. The hypothesis tested was that the discrepancies between control and experimental positive responses were merely chance. Table 3 shows that this hypothesis could be rejected with a high degree of confidence in every case ($P = < .02 - < .005$) of the four warming experiments. For the cooling tests, inspection of table 2 suffices to show that the chance hypothesis can again be rejected with a high degree of confidence.

Having ascertained that our pooled response data were probably not a chance result, we divided the positive warming response data for each species according to observer and using the Kendall sum method (Wilcoxon 1945) with emendations by Tukey (unpublished), tested the hypothesis that discrepancies between distributions of positive observations made by two observers working independently on the same group of animals were due to chance.

While credit for the method employed here must go to Wilcoxon (1945), the revised table of critical values which allows testing of unpaired data and the demonstration of the usefulness of this nonparametric small sample statistic is credited to Tukey (unpublished mimeo. from lecture No. 2 at Statistics Symposium Yale University, February 1950). There seem to be no assumptions about distributions in this method. Distributions rather than parameters (either measurement or enumeration data) are tested for similarity. The method, though ignored in many standard statistics references, is most useful for postexperimental analysis of odd or unexpected

TABLE 3

RESULTS OF χ^2 TESTS OF POOLED POSITIVE WARMING RESPONSE DATA

Species	Goldfish	Bullheads	Creek Chubs	Pumpkinseeds
χ^2	6.09	6.10	15.01	12.05
P	<.02	<.02	<.005	<.005
Decision	Reject chance	Reject chance	Reject chance	Reject chance

(One degree of freedom in all cases)

TABLE 4

SUMMARY OF TESTS OF COINCIDENCE OF WARMING RESPONSE DISTRIBUTIONS FOR THE DATA OF TWO OBSERVERS

Test for		Species			
		Goldfish	Bullheads	Creek Chubs	Pumpkinseeds
Total Positive Responses	Kendall Sums	24*	23	23	21
	Group Sizes	6,9	7,8	8,7	7,8
	Decision	accept	accept	accept	accept
Positive Responses < 0.1°C	Kendall Sums	21.5	12.5	23.5	21
	Group Sizes	6,9	7,8	8,7	7,8
	Decision	accept	marginal; significant at 8–9%	accept	accept
Mean Levels of Response	Kendall Sums	18.5	13.5	28**	20.5
	Group Sizes	6,9	7,8	8,7	7,8
	Decision	accept	marginal but > 10%	accept	accept

*For groups of 7 and 8 or 6 and 9, Kendall Sums above approximately 13 support the chance discrepancy hypothesis.

**A sum of this size for groups of 7,8 indicates complete coincidence of distributions.

behavior or of sidelines to the main purpose of the experiments and is also ideal for critical purposes.

In no case was the Kendall sum even near the 10 percent critical limit, indicating that the two independently obtained distributions of positive responses for each species overlapped to such a degree that the small discrepancies noted were quite likely chance results.

The warming responses under 0.1°C for each species were similarly divided according to observer and tested by the Kendall sum method. Only in the case of the bullheads did we differ significantly and then at only the 8 to 9 percent level. The peculiar resting behavior of this species may account for this difference of opinion.

The mean levels of response in °C were tested in a similar manner and no significant discrepancy between authors was noted although the bullhead distributions were close to the 10 percent significance level. With regard to the statistic used, it should be mentioned that while the Kendall sum method showed the bullhead measurement data to be near significance, a "t" test of the same data gave $P > .40$ indicating that the easily calculated Kendall sum may well be more reliable for critical examination of small samples with unknown distributions.

A summary of all of the Kendall sum tests is shown in table 4. Similar agreement between authors for cooling responses (not shown) was apparent by inspection. Both authors obtained a mean value of $-.12$°C for the goldfish and $-.24$°C for trout. Kendall sums were 14 for the mean levels of response of the two species subjected to cooling indicating nearly complete coincidence of response distributions (group sizes 6,5).

Between-species differences in levels of response were also examined. While the means in tables 1 and 2 may seem to indicate significance, all

species tested showed ability to discriminate warming of +.05°C and goldfish showed cooling responses at −.05°C, so that we were able to detect no major differences in the threshold temperature sensitivity between species. Thus, the means may well reflect differences in alertness and "intelligence" rather than temperature sense *per se*.

However, because the rainbow trout were not observed to respond to cooling of less than −0.1°C and pumpkinseed warming responses appeared to "pile up" near the lower limit we graphically analyzed (figure 2) the mean response data and statistically tested the differences in the between-species variance in levels of response. The hypothesis tested was that of equal variance between groups. Table 5 summarizes the results of the tests with "F" values for the range $F_{.975}$ to $F_{.025}$, indicated according to the number of degrees of freedom in each case.

In making the comparisons, all warming trials having mean levels of response above .32°C were arbitrarily eliminated because inspection of figure 2 indicates that these responses (two each in the goldfish, creek chubs and bullheads) were early experimental runs biased by learning factors. The elimination of such values makes our test of between-species variance a conservative one.

The fact that no mean values below ±.05°C are shown on the graph is of course an artifact inherent in our recorder, which could not be read accurately to nearer than .05°C. Note, however, that in all cases the values cluster somewhere beyond the ±.05°C measurement limit.

Table 5 shows that there is no significant difference in the variances of the two minnows' mean levels of response. When the variances of the two minnows are combined and tested against the bullheads the result is marginal at the .05 level. However, when the variances of all three of the former are combined and tested against the pumpkinseeds, the result is highly

TABLE 5

COMPARISON OF THE VARIANCES OF BETWEEN-SPECIES MEAN LEVELS OF RESPONSE

Group tested	Degrees freedom	S^2	F	Limits of F		Decision
				Upper	Lower	
Goldfish versus Creek Chubs	12,12	.0087 .0071	1.23	3.28	.305	Accept hypothesis of equal variances
Goldfish + Creek Chubs versus Bullheads	24,12	.0158 .0052	3.04	3.02	.394	Marginal
Goldfish, Creek Chubs & Bullheads versus Pumpkinseeds	36,14	.0210 .0022	9.55	2.70	.427	Reject
Trout versus Goldfish (Cooling)	10,10	.0099 .0031	3.19	3.72	.269	Accept*

*Can be rejected at .1 level.

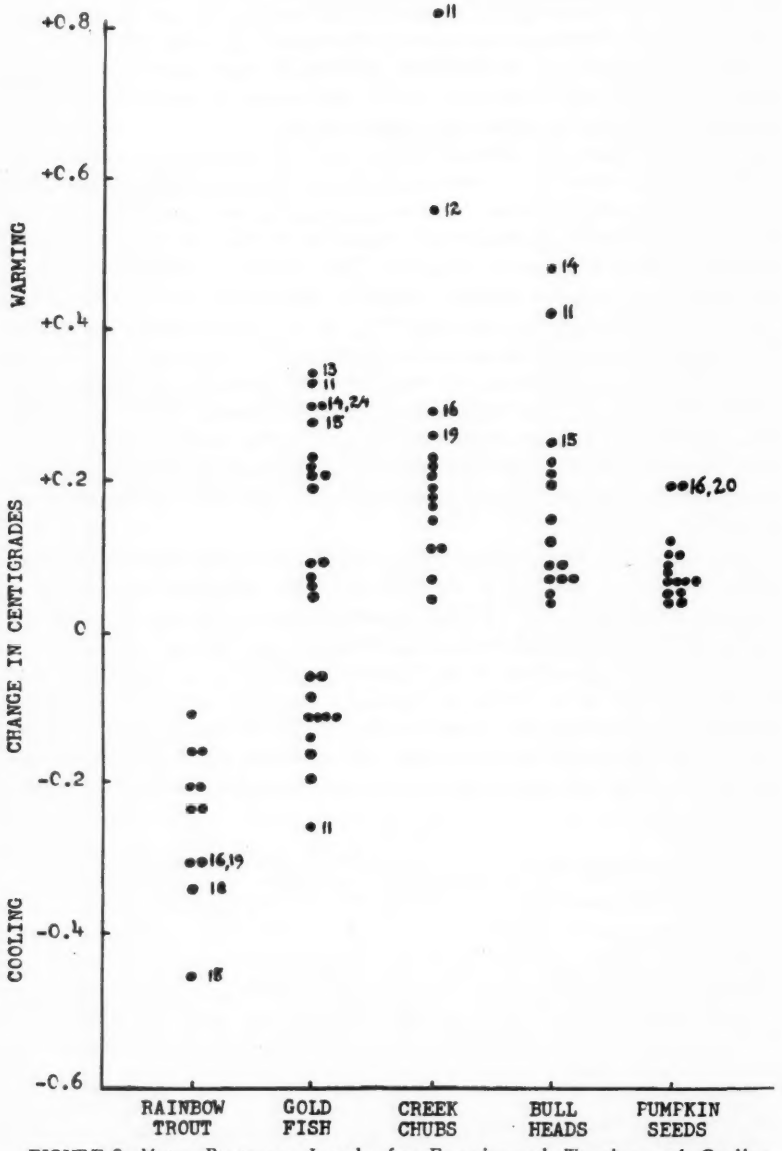

FIGURE 2. Mean Response Levels for Experimental Warming and Cooling. (Numbers indicate trial numbers, starting with #11, as in Tables I and II; see Section on Statistical Treatment of the Data for discussion).

significant. The samples for cooling are smaller and were not treated "conservatively," and, although not significant at .05 level, the variances of the trout and goldfish cooling response data are significant at the .1 level.

If one ignores the biased trials, as was done above, the data in figure 2, though incomplete for both warming and cooling for species other than the goldfish, appear to form a pyramid of decreasing variances when arranged by species in the usual phylogenetic order from left to right. This was unnoticed until after our experiments were terminated and first drafts of this paper written. As far as they go, the data seem to support our contention that the thresholds of temperature sensitivity are approximately the same for all species listed. However, the specific behavior patterns controlled largely by the temperature range of the experimental environment may well account for some of the disproportionment in variances.

Certainly not all of the species tested have the same temperature preferendum. During the trout cooling experiments temperatures at the start of a day's trials averaged 18 ± 1.0°C.

Thus, for the trout we were quite near the upper limit of the species' temperature preferendum. Lumping the tests of all species the temperature range was approximately 19 ± 2.5°C.

The general level of some forms of activity such as frequency of movements have been shown to be characteristic of the acclimation temperature for a given species Sullivan (1954).

While the data in figure 2 suggest the possibility that alertness to change in temperature increases with progress up the phylogenetic line in fish, our experiments were all run within a relatively narrow temperature range and though the animals were acclimated thoroughly for the experiments, we have not determined the preferendum for each of the species.

Therefore, we must leave the question open until performance data for both cooling and warming at several different temperatures including the preferendum temperature are available for each species.

DISCUSSION

The above observations as well as the fact that acuity of temperature perception increases with training, may lead one to believe that the very small temperature fluctuations which fish are actually capable of sensing are generally not reacted to. It was only when a small change became meaningful that we were able to reach, or perhaps only approach, the lower limit of temperature perception. The more rapid and the more pronounced the change in heat energy flow, the more immediate is the animal's reaction to it.

A rise or a fall of .2°C. within an hour as it occasionally occured in the controls, was apparently not a rapid enough change to be perceived by our trained fish. Reactions occurred when the changes up or down were at least .05°C./min.

Dijkgraaf (1940) mentions that fish have a "cold sense and warm sense" but he does not discuss the mechanism by which this discrimination might be achieved. Mammals have developed sensory endings which are differentiated with regard to their most effective ranges but since no histologic specialization of nerve endings in the fish skin has hitherto been detected

and no skin-nerve preparation isolated, this question can, at present, only be dealt with by speculation.

The simplest arrangement would be a set of free nerve endings—and they are present in fish skin in great numbers—which are temperature sensitive and respond to changes in environmental heat flow by either increased or decreased discharge rates comparable to the Ampullae of Lorenzini in elasmobranchs (Sand, 1938), and also to the nerves of the pit organ in the rattlesnake (Bullock and Faulstick, 1953). Granit's (1955) discussion on peripheral principles of organization in sensory perception, especially with reference to thermo-receptors in homoio and poikilotherms lends further theoretical support to our interpretation. Our observations on adaptation with warming and subsequent cooling of trout and to some extent the goldfish also point in this direction.

As an alternative, one might postulate change receptors whose slight responses to "thermal background noise" are not reacted to. Upon a greater change in heat flow, whether up or down, the increase in volleys fired alerts the animal, somewhat comparable to certain retinal or kinesthetic receptors.

This mechanism of temperature perception in fishes would leave unexplained the animal's ability to distinguish an upward from a downward change in environmental heat flow unless one were to also assume the presence of one or more other elements which enable the animal to differentiate heating from cooling.

It has been shown in the past that fish have temperature preferenda and this in itself implies the capacity of temperature differentiation. The adaptive significance of acute sensitivity to temperature changes may well be that it enables the fish to follow temperature gradients closely and remain narrowly within an isothermal water layer.

SUMMARY

The mechanism involved in temperature perception, namely, areal or spatial summation from individual small skin fibers enables the fish to sense rapid temperature changes of about .05°C.

Although the "thermal background noise" in even a "stable" aquarium environment must often reach this level of fluctuation in heat energy flow such small changes are ordinarily ignored.

Acuity of perception increases on training as small thermal changes become meaningful.

The mechanism by which such fine discrimination can be achieved, is discussed.

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ANTIGENIC RELATIONSHIPS AMONG FEULGEN POSITIVE CYTOPLASMIC PARTICLES IN PARAMECIUM[1]

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Three types of microscopic Feulgèn positive cytoplasmic particles have been found among various related strains of *Paramecium aurelia.* Kappa particles are distinguished by the fact that animals containing them liberate a poison, "paramecin," into the fluid in which they live; paramecin normally kills sensitive animals (Sonneborn, 1943). "Mu" particles are known (Levine, 1953; Siegel, 1954) to cause sensitives to die after they have conjugated with animals, "mate-killers," carrying the particles; however they are not responsible for the production of poisons detected in the culture medium. Finally, Hanson (1954) discovered particles which are mutants of kappa and whose presence induces no recognizable effects on other cells; they are only detected cytologically. Since these types of particles are similar in numbers per cell, size, shape, and staining reactions they are evidently related. Furthermore, the origin and evolution of the particles has remained a matter of much speculation especially among those concerned with their meaning for models of gene action and cellular differentiation. (See Beale, 1954 for a recent discussion.) In view of these facts it was of interest to investigate the antigenic relationships between the particles.

Sonneborn (1956a) has shown that *P. aurelia* may be divided into twelve sexually isolated varieties or physiological species. Kappa has been found in varieties 2 and 4; mu has been found in variety 8 and pi in variety 4. Recently killers (presumably containing kappa) have been reported from varieties 6 and 8 (Sonneborn, 1956b). On the basis of their lethal effects, the mu particles of variety 8 are quite different from the kappa and pi particles of varieties 2 and 4. The kappa particles of varieties 2 and 4 seem very similar, for although each strain causes characteristic prelethal effects on the sensitives, several of the variety 2 and 4 strains produce almost identical effects in sensitive paramecia. On the other hand, strains belonging to varieties 4 and 8 are closely related, for they will mate freely (but give inviable progeny) and share various other characteristics. Variety 2 is unrelated to either; intervarietal matings do not occur and in other respects this group of strains appears quite different. It seemed that a study of antigenic relationships among the various types of particles might bear on the problem of the evolution of the strains of paramecia in which the bodies occur.

[1]This work was supported by grants from the National Science Foundation and the Phi Beta Psi Sorority.

MATERIALS AND METHODS

The following strains of *P. aurelia* were used: stocks G and 50 are killers and belong to variety 2; Gml is a mutant killer derived from stock G; stocks 51 and 169 are killers and belong to variety 4; De-51ml is a mutant killer obtained from stock 51; 51ml is a mutant obtained from stock 51 and contains pi particles; stocks 130, 131, and 138 are mate-killers and belong to variety 8.

The standard methods described by Sonneborn (1950) for the study of *P. aurelia* were employed. The fact that kappa particles are not distorted in fresh breis of killer paramecia and can be readily identified and studied with the bright phase-contrast microscope is known (Preer and Stark, 1953); similar techniques were found suitable for observations on mu and pi bodies.

To avoid confusing the particles with bacteria recently ingested by the paramecia and thus present in the breis, the animals were cultured on a bacteria-free medium consisting of 0.25% yeast extract and living cells of a strain of *Saccharomyces cerevisiae.* The particles present in paramecia cultured in this manner were then satisfactorily identified. A few strains could not be established in this yeast medium; these were freed of bacteria by passing them through a series of washes in sterile 0.25% yeast extract; after six hours and four to eight transfers the bacteria had disappeared. Once familiarity with the various particles had been attained, the presence of bacteria no longer hampered identifications and the investigations were continued using bacteria as a food source for the cells.

In order to prepare suitable antisera, mass cultures of paramecia containing particles were grown in bacterized culture medium with sufficient food to permit a slow rate of reproduction. Under these conditions a high intracellular population of particles is assured. The cells were concentrated and injected into rabbits using previously described techniques. To test the antisera thus produced, about twelve paramecia were isolated on a slide in a very small volume of culture fluid together with an equal amount of homologous undiluted antiserum. A thin coverslip was then placed on top of the drop and the cells were crushed carefully by applying pressure to the coverslip. Observations with the phase-microscope disclosed that the particles had flowed free of the disrupted cells and were agglutinated by homologous antisera. The agglutination occurs rapidly: in a few minutes the particles stick to one another in a random manner, without respect to obvious differences in size or morphology (Preer and Stark, 1953). Repeated trials always provided concordant results. When the sera were diluted by a factor of 10 the agglutination phenomenon disappeared. Control sera were prepared by injecting into rabbits cells isogenic with those mentioned above but lacking kappa; such sera failed to cause particles to clump and thus attest to the specificity of the agglutination reaction.

RESULTS AND DISCUSSION

The results are presented in Table 1. The data illustrate the fact that an

TABLE 1

THE REACTIONS OF DIVERSE PARTICLES IN THE PRESENCE OF HOMOLOGOUS AND HETEROLOGOUS ANTISERA. THE PLUS SIGNS INDICATE AGGLUTINATION; THE MINUS SIGNS INDICATE A FAILURE TO AGGLUTINATE; THE PLUS-MINUS SIGNS INDICATE WEAK REACTIONS IN WHICH A FEW PARTICLES ARE AGGLUTINATED AND WHICH MAY GIVE NEGATIVE TESTS.

Particles \ Antisera		Var. 2		Var. 8			Var. 4	
		G	Gml	130	131	138	De-51ml	51ml
Var. 2	G Kappa	+	+	−	−	−	−	−
	Gml Kappa	+	+	−	−	−	−	−
	50 Kappa	+	+	−	−	−	−	−
Var. 8	130 Mu	−	−	+	+	+	+	+
	131 Mu	−	−	+	+	+	+	+
	138 Mu	−	−	+	+	+	±	±
Var. 4	De−51ml Kappa	−		+	+	+	+	+
	51ml Pi	−		+	+	+	+	+
	51 Kappa	−	−	+	±	+	−	+
	169 Kappa	−		+	+	+		+

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antiserum prepared against the particles found in a given stock will agglutinate the homologous particles and will also react with heterologous particles obtained from stocks belonging to the same variety. Thus these cross-reactions make it evident that particles *known* to have arisen by mutation from a "wild-type" particle possess antigens in common with their progenitors. The only exception to this pattern is the failure of anti-de-51ml serum to agglutinate kappa from stock 51, a remarkable finding in view of the fact that these kappas are agglutinated by sera prepared against particles from variety 8! However, this observation is paralleled by Hanson's (1954) description of pi particles discovered by him in stock 51 and which did not show antigenic cross-reactions with kappa. Thus, although related particles sometimes fail to show cross-reactions, it is reasonable to assume that the cross-reactions among particles of *unknown* origin indicate a recent common evolutionary origin.

Of considerable interest is the consistent antigenic difference between particles found in stocks belonging to varieties 2 and 4, and 2 and 8 on the one hand, and the similarity between particles found in stocks belonging to varieties 4 and 8 on the other. Thus kappa of variety 4 would appear more closely related to mu of variety 8 than to kappa of variety 2! The data suggest either that the particles of variety 2 stocks are distantly related to the others or that they are of independent origin. In either case the comparison of the antigenic cross-reactions of the particles indicates a parallel evolution for particles and the stocks in which they are found because, as mentioned earlier, varieties 4 and 8 are closely related and neither shows affinities with variety 2.

The results of previous studies (Dippell, 1950; Levine, 1953; Hanson, 1954, 1956) document the mutability of kappa and mu to forms which elicit novel effects on sensitive cells and to particles with a markedly altered maximal reproductive rate. The results of the present investigation establish that such changes may be independent of variations in antigenic specificity and so provide additional evidence for the genetic complexity of these bodies. Finally, these observations make more practical an attack on the mechanism of reproduction of the particles; if particles differing in two characteristics are combined in a single paramecium, allowed to reproduce, and then sorted out again, the occurrence of any genetic recombinants which might occur could be detected.

The morphological complexity of kappa (and related particles) was discovered when new techniques for their study became available (Preer and Stark, 1953). Thus it was shown that a certain portion of the Feulgen positive cytoplasmic bodies in a killer cell function as paramecin and that these particular kappas contain one or more unique refractile granule (Preer, Siegel, and Stark, 1953). The property of specific antigenic agglutination indicates that the surface of the particles contain specific protein or polysaccharide. Further experiments combining the techniques now available with enzymatic digestion should disclose the chemical nature of the outer surface and may relate this to the biologic properties of the various particles.

The question as to whether these cytoplasmic particles are descendants of foreign organisms parasitic on paramecia or, on the contrary, are of intrinsic origin and a part of the hereditary apparatus of the cells (i. e., plasmagenes) has intrigued biologists since their discovery. From Beale's (1954) recent summary of the facts, it is clear that no definite answer can presently be given to this problem. Although he concludes that there is no evidence which conflicts with the view that the particles have "affinities with other free-living or parasitic organisms" (page 75) it is equally clear that the data do not rule out an intrinsic origin. The studies of kappa and its relatives reported here and elsewhere reveal that these particles possess antigens and other highly characteristic structures and specificities which have no connection with the hereditary determinants of the paramecium cell. It is reasonable to expect that future work will further define the special features of these particles and so will serve to distinguish them from other biological units.

SUMMARY

Kappa, mu and pi particles were shown to be antigenic; the pattern of serologic cross-reactions suggests a parallelism between the evolution of the particles and the stocks of paramecia in which the particles are found. Alterations in the biologic activities of the particles are usually independent of marked changes in antigenic specificity but two exceptions to this are noted. The significance of these facts for further investigations of the biology of these particles is discussed.

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AN EXTREME KARYOTYPE IN AN ORTHOPTERAN INSECT

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In the course of investigation on the chromosomes of Uruguayan Orthoptera belonging to the family Acrididae, a brachypteran form was found among the specimens of the genus Dichroplus that possesses a karyotype completely different from those known in any of its close or remote relatives. (After this manuscript had been written, this form was classified as a new species *Dichroplus silveiraguidoi Lieb* (in Lit.) by Dr. J. Liebermann, División Zoologia Agrícola, Ministerio de Agricultura, Buenos Aires, Argentina.)

Only one male individual was available for cytological study among several collected in Cerro Batovi, Rivera, Uruguay. Gonads were fixed in alcohol-acetic acid (3:1) and treated by Feulgen squash technique. Very minute pieces were taken out, squashed, and permanent mounts were made in cedar wood oil. The photomicrographs were taken with Leitz Apo.Ob.90 and Oc. × 10.

RESULTS

Detailed observation disclosed all stages of spermatogenesis, from spermatogonia to mature spermatozoa.

Entirely unexpectedly, the diploid number of chromosomes was found surprisingly low in relation not only to those in other species of the family Acrididae but to all so far studied orthopteran insects. The diploid set consists of 2n = 8 chromosomes, (figs. 1, 2, 3). The four chromosome pairs will be called A, B, C and D. Chromosome A is metacentric or V-shaped, with limbs nearly equal in length. B is submetacentric with unequal limbs. C is acrocentric, its centromere being situated very close to the proximal end; the morphology of these three elements is very well shown in figure 17. The D pair is composed of two unequal acrocentric chromosomes differing in length in a ratio of 1 to 3. Since in this case the odd chromosome, which represents the sex chromosome in the majority of the Acrididae, is not present and the D pair exhibits a differential behavior during meiotic prophase and metaphase, this pair is believed to represent the X-Y sex chromosomes.

The spermatagonial chromosomes are radially arranged on the equatorial plate excepting the smallest element (Y chromosome) which sometimes occupies the center of the plate. (fig. 2).

The three bivalents and the heteromorphic X-Y pair can be seen clearly at the end of pachytene (fig. 4). The metacentric chromosomes show a higher number of chiasmata. The acrocentric bivalent, in spite of being a

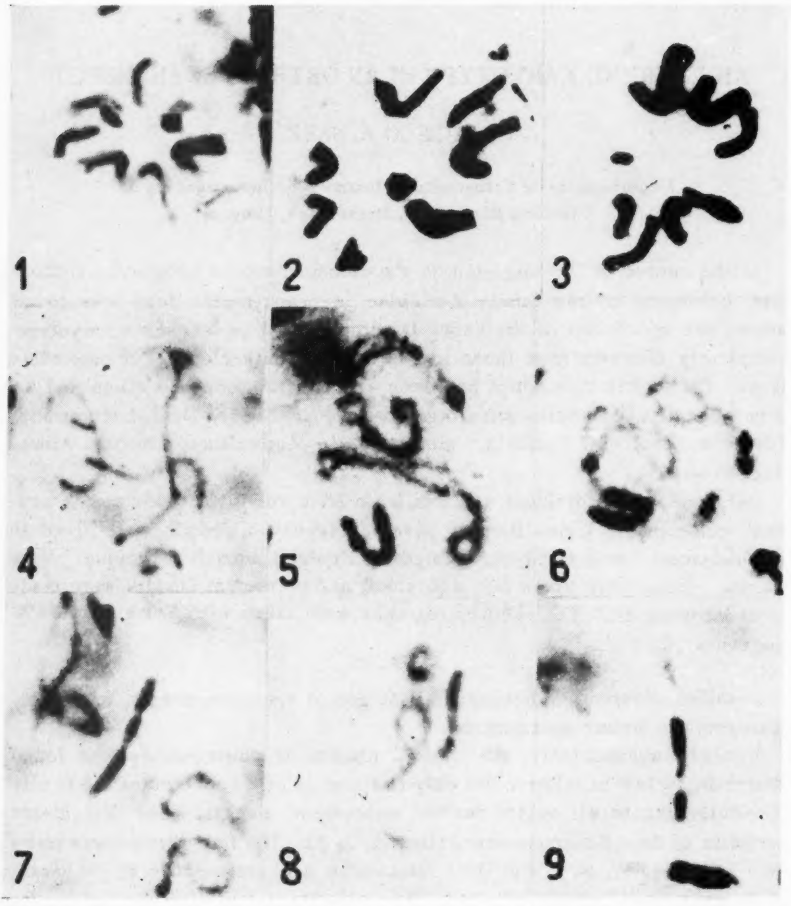

FIGURE 1, 2, and 3: Polar view of spermatogonial metaphase showing the 8 chromosomes and in Fig. 2 the morphology of the four pairs A, B, C and XY.

FIGURE 4: End of pachytene.

FIGURE 5, 6, 7 and 8: Diplotene stage showing the configuration and behavior of the bivalents and the XY pair.

FIGURE 9: The unequal bivalent XY at the end of diplotene.

fairly long chromosome, displays a tendency to the anular shape with a proximal and a distal chiasmata located near the ends. Other characteristics such as localization, chiasma frequency, terminalization, will be reported when more material becomes available.

At this stage the unequal pair is composed of two chromosomes associated by their distal ends, forming a constriction, (figs. 5, 6, 7, 8). Its configuration is that of a long rod-shaped element, composed of two different heterochromatic regions: a positive heteropycnotic, and a granular less

condensed negative heteropycnotic segment, similar to the other euchromatic bivalents (figs. 6, 7, 8). In some slides, this last euchromatic region may show a second constriction (figs. 7, 8). During the pachytene and diplotene, the X-Y pair is thus constituted by the original X chromosome, probably associated by a terminal chiasma with one autosome that would correspond to the Y chromosome (fig. 9). It seems probable, that due to a series of structural changes, the homologous segments undergo a process of shortening until they are reduced to the distal regions of these chromosomes.

In polar and side views the first meiotic metaphase shows the configuration and position of the bivalents on the spindle (figs. 10 to 14). The metacentric bivalents have the annular aperture parallel to the axis of the spindle while in the acrocentric bivalents this aperture is perpendicular (figs. 12, 13). The X-Y pair is inserted perpendicularly with respect to the equatorial plane so that both elements are held under tension and are associated by a terminal chiasma with the proximal ends directed toward the poles (figs. 10, 12, 14). Sometimes the position of the X chromosome is different since it lies along the equatorial plane perpendicularly to the axis of the spindle while the Y is at a right angle, parallel to the axis (figs. 11, 13).

During anaphase the homologous chromosomes segregate toward the respective poles so that the reduction takes place for all chromosomes at this first meiotic division (figs. 15, 16). The behavior of the X-Y pair seems to indicate that a terminal chiasma is formed between both chromosomes thus assuring the segregation of the homologue in the first meiotic anaphase.

The second meiotic division gives rise to two kinds of spermatids, half with the X (fig. 17), and half with the Y chromosome (fig. 18). This is easily seen in polar views of the metaphases of this division.

DISCUSSION

A great majority of species of acridids possess the presumed ancestral karyotype of 2n (♂) = 23 acrocentric chromosomes. The situation in the acridids differs, consequently, from that in Drosophila. In the latter, the putative ancestral karyotype of 2n = 12 acrocentric chromosomes has become altered in a variety of ways in many species.

The case of Dichroplus stands out because in this form not only the number of chromosomes but also the number of chromosomal arms, the so-called "fundamental number," has become radically altered. Instead of the 23, Dichroplus has only 12 chromosome arms, in other words 11 acrocentric chromosomes appear to be missing from its karyotype. The derivation of the Dichroplus karyotype from the presumed ancestral one must, therefore, have involved not only centric fusions of the ancestral acrocentric chromosomes but also loss of several chromosomes as independent units.

The most likely interpretation of the formation of metacentric from acrocentric chromosomes is centric fusion. As pointed out by White (1954), centric fusions are not selected against in heterozygous condition in natu-

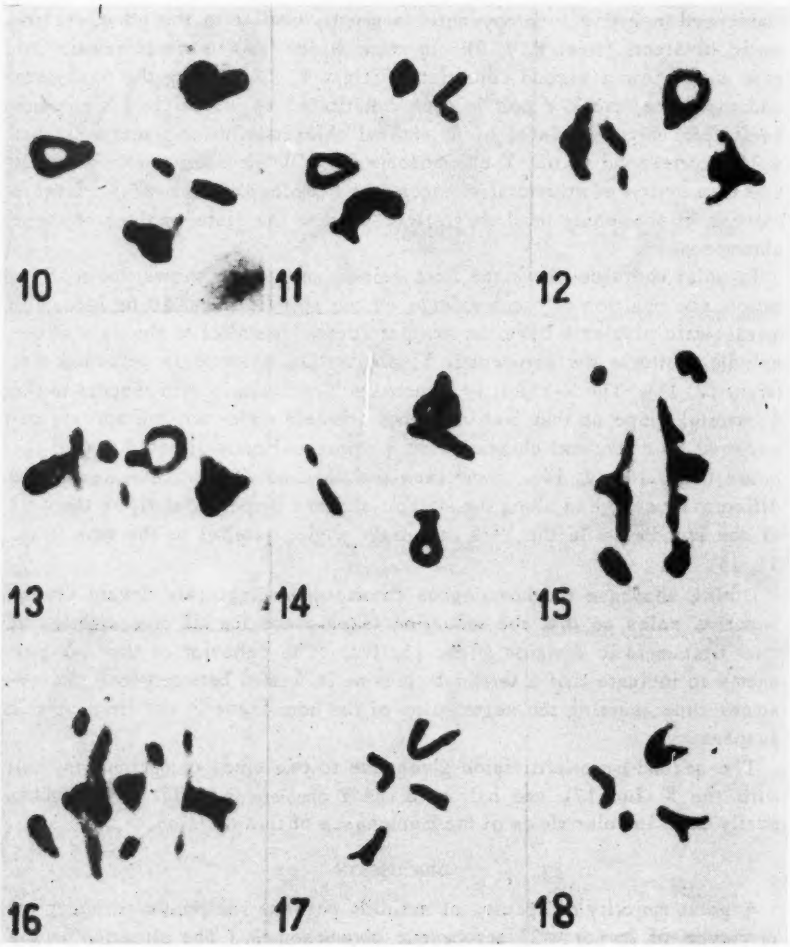

FIGURE 10, 11: Polar views of first meiotic metaphase showing the behavior of the XY unequal pair.

FIGURE 12, 13 and 14: Side views of first meiotic metaphase.

FIGURE 15: Meta-anaphase of first meiotic division.

FIGURE 16: The XY pair in anaphase and the A B and C bivalents in late metaphase.

FIGURE 17: Polar view of second meiotic metaphase with the X chromosome.

FIGURE 18: Polar view of second meiotic metaphase with the Y chromosome.

ral populations. The situation may, however, be quite different with the "loss" of the chromosomal units. This process involves translocation of the material of one chromosome into another member of the set, and a loss of the centromere with the adjacent heterochromatic region. Translocation heterozygotes are likely to have a lowered fertility.

Apart from Dichroplus, this process has apparently taken place in another acridid, *Dactylotum bicolor*, which has the lowest chromosome number which was known among grasshoppers before the discovery of Dichroplus, namely 17 acrocentric chromosomes in the male (Helwig 1942, Powers 1942). Three pairs of chromosomes have disappeared as independent units in the evolution of this form from the ancestral stock. Since in Dactylotum the chromosome number is reduced below the basic number, and yet all the chromosomes remain acrocentric, translocation must also have taken place. Other cases of the same sort involve the reduction of the chromosome number by means of formation of double-length chromosomes in certain species of Drosophila, namely *D. spinofemora*, *D. tranquilla*, and *D. testacea* (Wharton, 1943). This must have occurred by translocation of the material of one chromosome arm, minus its centromere, onto another rod, or by fusion of two rods followed by a pericentric inversion shifting the centromere to a subterminal position.

Among the orthopterans double-length chromosomes have not yet been found, at least to judge by the relative sizes of the chromosomes in different species. If such a change has occurred it is not possible to verify it by the size of the metaphasic or anaphasic chromosome. However, the possibility that a process of linear contraction of the chromosome has occurred can not be discarded. Whether such a contraction may be related to the mechanical adaptation of the chromosome to the spindle system remains to be proven.

So far it has not been possible to establish the exact mechanism by which the elimination of the 11 chromosomes in Dichroplus has occurred. In order to get a better insight into this problem it will be necessary to make a detailed comparative study of the relative dimensions of the chromosomes in the caryotypes of different species of Dichroplus. This study is now being pursued (Saez, 1956).

SUMMARY

The unique case of an orthopteran acridid of the genus Dichroplus (brachypterous form) with 2n (♂) = 8 chromosomes is described. The karyotype is composed of two pairs of metacentric, one pair of acrocentric and an unequal pair of chromosomes X-Y.

The morphological characteristics and the behavior of the three pairs of bivalents and the XY pair can be followed during the prophase and metaphase. All bivalents achieve a segregatory division of the homologues in the first meiotic anaphase. The second meiotic division gives rise to two types of spermatids one with the X chromosome and the other with the Y. The probable mechanisms involved in the production of this extraordinary cytological evolutionary transformation are discussed.

I want to express my gratitude to Prof. T. Dobzhansky for the critical reading of the manuscript and to Prof. A. Silveira for having provided the material. I also wish to thank Mr. E. Valdes for technical assistance.

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GENETIC STUDIES IN AEDES

I. The Distribution of Polytene Chromosomes in *Aedes aegypti*

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This paper discusses the tissues of *Aedes aegypti* which contain cells with polytene chromosomes and correlates their distribution with stage of development and embryonic germ layer.

The first report of polytene chromosomes in mosquitoes was by Bogojawlensky (1934) who reported their occurrence in the salivary glands, malphigian tubes, midgut, and the anterior portion of the hindgut of *Anopheles maculipennis* larvae. Subsequently, cytogenetic studies have been made on the polytene chromosomes of the larval salivary glands in Anopheles (Frizzi, 1947 and other papers) and *Culex pipiens* (Kitzmiller and Clark, 1952). Berger (1936) has discussed the polytene chromosomes of the larval midgut of *C. pipiens*. Sutton (1942) and Sengün (1948) are the only authors who have considered polyteny in the genus Aedes extensively. Sutton's investigations showed that polytene chromosomes occur in cells of the malphigian tubes of larvae, pupae, and adults, and in the gastric caecae, midgut, and salivary glands of the larvae of *A. aegypti*. Sengün studied the development of polytene chromosomes in the salivary glands, crop, midgut, malphigian tubes, and rectum of an Aedes species and a Culex species as well. He reported that in the imago, chromosomes were not seen in midgut or salivary gland preparations, but were in those of the malphigian tubes. He also stated that complete development of polytene chromosomes was traced in the salivary glands, crop, and midgut of the Culex species, but only in the salivary glands of the Aedes species.

In the present work, the nuclear types of a number of tissues taken from fourth instar larvae, pupae, and adults were investigated. This made it possible to determine whether or not the cells of different tissues and organs gained or lost polytene chromosomes during different parts of the life cycle.

MATERIALS AND METHODS

Tissues, obtained from *A. aegypti* reared at Fort Detrick, were dissected fresh into distilled water or *Aedes aegypti* Ringer solution (Bradford and Ramsey, 1949). Both of these proved satisfactory for cytological purposes. The tissues were then smeared whole in aceto-orcein or Belling's iron aceto-carmine. Both stains generally give good chromosomal detail, but the aceto-carmine stained the cytoplasm as well, while the aceto-orcein did not. The preparations were generally studied fresh, but could be used for several days without drying when sealed with Permount and refrigerated at 10°C.

RESULTS

In *A. aegypti*, polytene chromosomes were found primarily in cells of the alimentary epithelia of the larva. As shown in Table I, the epithelia included those of the salivary glands, gastric caecae, midgut, malphigian tubes, and rectum. In addition, these chromosomes were also found in the large cells of the anal papillae of the larva. During pupation the tissues and organs with polytene-chromosome-containing cells became histolyzed and were either rebuilt or lost entirely with one exception. The cells of the malphigian tubes were not histolyzed during pupation and smears of pupal and imaginal tubes showed large nuclei with definable chromosomes. In the midgut, on the other hand, the large nuclei of the larva became shrunken and deformed during pupation and the chromosomes which they contained lost their definition. They were replaced by many smaller nuclei which did not show polytene chromosomes when stained.

DISCUSSION

K. W. Cooper (1938), in a review of the existing data on polyteny, pointed out that, in general, all larval tissues destined to undergo histolysis during metamorphosis appear to increase by growth in cell size rather than cell division. This is the case with the large polytene-chromosome-containing cells of the larva of *A. aegypti*. These histolyze during pupation and are replaced by new cells which are not polytene. Thus, it appears that in *Aedes aegypti* polytene chromosomes are a characteristic of the larva just as distinctive as the anal papillae. The only cells which make exception

TABLE I

DISTRIBUTION OF POLYTENE-CHROMOSOME-CONTAINING CELLS IN TISSUES AND ORGANS OF *AEDES AEGYPTI*. THE SYMBOLS USED ARE: P = POLYTENE; N = NOT POLYTENE; A = TISSUES ABSENT.

Tissue	4th instar larvae	Imago
Salivary glands	P	N
Oesophagus	N	N
Proventriculus	N	N
Gastric caecae	P	A
Midgut	P	N
Malphigian tubes	P	P (♀)*
Hindgut	N	N
Rectum	P	N
Anal papillae	P	A
Dorsal diverticula	A	N
Nervous tissue	N	N
Fat "body"	N	N
Imaginal discs	N	A
Thoracic muscle	N	N
Vesicula seminalis	–	N
Accessory glands	–	N
Ovarian duct	–	N
Nurse cells	–	N
Follicular epithelium of ovary	–	N

*Polyteny is not definitely established in the males.

to this statement are those of the malphigian tubes. These cells do not histolyze during pupation, and in the adult female, at least, they are definitely polytene. Polyteny in the malphigian tubes of the adult has been reported in Aedes and Culex by Sutton (1942), and also in Rhynchosciara by Dreyfus et al. (1951). The latter authors also state that a portion of the vesicula seminalis of the adult male is composed of polytene cells. Stalker (1954) in a cytological survey of the ovarian nurse cells of 190 species of Diptera belonging to 40 families showed that banded polytene chromosomes were present in 16 species belonging to the families Cecidomyidae, Empidae, Dolichopidae, Lonchopteridae, Helomyzidae, Agromyzidae and Tachinidae. Therefore, it is evident that the degree to which polyteny is restricted to the larva varies from one group to another.

In *A. aegypti*, the tissues in which polytene-chromosome-containing cells are found are of ectodermal and endodermal origin according to Snodgrass (1935). The ectodermal tissues and organs include the salivary glands, malphigian tubes, rectum, and anal papillae. Those of endodermal origin are the gastric caecae and midgut. In *Drosophila virilis*, however, Makino (1938) records polytene chromosomes from muscle and fat body cells which are in tissues of mesodermal origin. Thus, in the Diptera generally polyteny may arise in cells of any one of the three germ layers.

The distribution of polytene chromosomes in tissues of other Diptera is frequently similar to that in *A. aegypti*, but there are important exceptions. Melland (1942) records them from nerve cells of Chironomus larvae, and Heitz and Bauer (1933) have found them in sporadic brain cells of the larvae of *Bibio hortulanus*. In *Drosophila virilis* larvae, Makino (1938) has seen polytene chromosomes in ganglion, muscle, fat, and tracheal cells. Cooper (1938) records them in addition from the hypodermis of Dipteran larvae.

Whether polyteny will prove to be a primarily larval phenomenon in Diptera other than *A. aegypti* is still open to question. Most of the literature referred to discusses polyteny in larval cells only. This seems to be indirect evidence that polyteny is primarily a larval phenomenon in the Diptera. However, the fact that polyteny does occur in the adult malphigian tubes, ovarian nurse cells, and vesicula seminalis of various genera shows that polytene cells can and do function in the adult in certain instances.

SUMMARY

Polytene chromosomes were observed in *Aedes aegypti* only in cells of ectodermal and endodermal origin. In larvae they were found in certain cells of the alimentary canal, its associated tissues, and the anal papillae. During pupation all tissues containing polytene chromosomes, except the malphigian tubes, were histolyzed and rebuilt or lost entirely. In adults polyteny was evident only in the malphigian cells of the females.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

WHAT IS "ENVIRONMENT"?

In the article "What is an adaptive trait?" (American Naturalist, 90:337), Professor Dobzhansky wrote: "The basic postulate of the modern biological theory of evolution is that adaptation to environment is the guiding force of evolutionary change." It seems to the writer that before we can discuss adaptive traits we must decide what is being adapted to. The term "environment", like "natural" for which it is a synonym in "natural selection", is a big word, subject to such enormously different interpretations as to be useless without definition.

On the one hand, "environment" may be thought of as ending at the organism's skin, as distinct from the organism, autonomous in itself. On the other hand, "environment" may be regarded as the organism's "environs", to some extent organized around and conditioned by the organism, inseparable from it. Environment has been thought of as including the spider's web, the bee's hive, cities of men, the wills, intelligences, and purposes of organisms. Darwin's sexual selection and J. M. Baldwin's organic selection both assume self-direction in the organism as factors in evolution distinct from control by forces external to the organism. Belief in "natural selection" rests largely on man's ability to guide evolution of domestic breeds. Species differences in flowers seem to be accounted for chiefly by preferential pollination by insects.

"Environment" and "natural" are seldom defined by present-day Darwinians. The "Origin of Species" offers little but confusion to the seeker after a clear definition. If the terms mean anything in Darwinism, not everything and therefore nothing, we seem bound to apply the first interpretation above. But if we entirely exclude organism choice as a factor in evolution, the key analogy with man's selection falls through. Vast areas of evolution in addition to human phenomena are inexplicable.

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January 7, 1957

THOMAS A. MORRILL

REPLY TO MR. MORRILL

Mr. Morrill asks for a definition of the "environment" to which organisms become adapted in the process of evolution. As with some other funda-

mental concepts of biology (life, gene, individual, species), an attempt to produce a formal definition would land us in a morass where no simple idea can be conveyed without endless quibbling and hair-splitting. Fortunately, the situation is not as bad as some strict logicians imagine. For example, although a valid general definition of what is an individual is hard to come by, one is seldom in doubt about what is a human individual and what is not one.

In the broadest sense, the environment is the whole universe. Such a broad concept would, of course, be worthless in biology; all organisms inhabit the same universe, but they often stand to it in different relations, even if they are close neighbors in the spatio-temporal continuum. Thus, *Drosophila pseudoobscura* and *Drosophila persimilis* fly together in the Transition Zone of the Sierra Nevada of California, but the former species is relatively more active in the evening and the latter in the morning. Their environments are, accordingly, not wholly identical. It would be equally unsatisfactory to restrict "environment" to physical factors, such as temperature, humidity, light, soil, and chemical composition of water of or food. Biotic factors are obviously important, and often decisive, components of the environment. This is especially evident in tropical rainforests, with their often rigorous biotic but uniformly favorable physical environmental constituents; conversely, arctic, alpine, and extreme desert habitats (such as the Egyptian Sahara and the dry coasts of Chile and Peru) have environments in which almost anything which is able to survive the climatic rigors does so.

Furthermore, as emphasized especially by Andrewartha and Birch (The Distribution and Abundance of Animals, 1954), every individual in a Mendelian population is part of the environment of other individuals. To an evolutionist, perhaps the most striking recent demonstration of this proposition is given by the findings of Lewontin (Evolution, 9:27) and Levene, Pavlovsky, and Dobzhansky (Evolution, 8:335), which show that the adaptive values of certain genotypes in Drosophila are modified by the presence in the same cultures of other genotypes of the same species. Interactions between organisms and environments are often reciprocal; a culture medium inhabited by a clone of bacteria or by a strain of Drosophila is evidently not like this medium was before the inhabitants were introduced.

The only sense in which environment and natural selection may be "ending at the organism's skin" is that the so-called internal environment and genotypic environment are really different phenomena from the "external" environment, and their names are metaphors which become misleading if taken too literally. Otherwise, the environment may be what the organism chooses it to be, within limits. Animals with highly developed nervous systems move freely from less suitable to more suitable environments. Man's genetic endowment enables him to control his environments and deliberately to create new ones of his invention and choosing. Realization of the simple fact that this ability is genetically controlled uncovers the

error of the widespread and oft repeated notion that natural selection no longer operates in modern mankind (see Dobzhansky and Allen, American Anthropologist, 58:591). In its simplest form, natural selection is adequately symbolized as a sieve which lets some genotypes go through but retains others. However, natural selection in its more complex forms becomes a sequence of challenges and responses, the workings of which grow less and less determinate, and consequently more and more creative (Dobzhansky, Atti IX Congr. Internaz. Genetica, 1:435).

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January 9, 1957

THEODOSIUS DOBZHANSKY

ERRATUM

In article by N. N. Kuznezov, Vol XC page 350, line 2 for "latitudes 37° and 40° N." read "latitudes 37° and 46° N."